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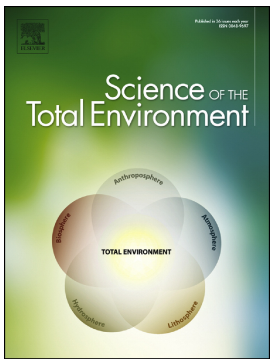

Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees, bee bread and honey: Beehive exposure assessment

A. Villalba, M. Maggi, P.M. Ondarza, N. Szawarski, K.S.B. Miglioranza

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Influence of land use on chlorpyrifos and persistent organic pollutant levels in honey bees, bee bread and honey: beehive exposure assessment

Villalba A.[1,2], Maggi M.[2], Ondarza P. M.[1], Szawarski N.[2], Miglioranza K. S. B.[1*]

[1]Laboratorio de Ecotoxicología y Contaminación Ambiental. Instituto de Investigaciones Marinas y Costeras (IIMyC), FECYN, UNMDP-CONICET. Funes 3350. 7600 Mar del Plata, Argentina.
[2]Centro de Investigación en Abejas Sociales (CIAS). Laboratorio de Artrópodos - Grupo Acarología y Entomología. Instituto de Investigación en Sanidad, Producción y Ambiente (IIPROSAM). CIC-UNMdP. FECYN, UNMDP. Funes 3350. 7600 Mar del Plata, Argentina.

Highlights

- OCPs, PCBs and PBDEs residues were found in honey bee, bee bread, honey, soil and flower samples.
- Chlorpyrifos was found in all samples from all sampling sites and periods.
- Honey bee samples were the most contaminated matrix of the beehive.
- Land uses and seasonal variations have affected directly the levels of pollutants observed in the beehive matrixes.

*** Corresponding author:** Karina S.B. Miglioranza. Laboratorio de Ecotoxicología y Contaminación Ambiental, IIMyC (CONICET).Universidad Nacional de Mar del Plata, Funes 3350, Mar del Plata (7600), Argentina. Tel.: +54 223 475 2426 ext 233
E-mail address: kmiglior@mdp.edu.ar

Abstract

This work reports the spatial and temporal variations on the dynamics of OCPs, PCBs, PBDEs and chlorpyrifos in honey bee, bee bread and honey samples, as well as soil and flowers from the surrounding areas, considering, different land uses. Honey bee samples showed the highest pollutant levels, with a predominance of the industrial contaminants over pesticides. Chlorpyrifos showed the highest concentration during the application period in almost all samples from the soybean field (S2), in concordance with its current use. By other hand, the recalcitrant compounds such as, DDTs, BDE #47 and also light PCBs exhibited the highest levels in beehive samples from the field adjacent to urban disposal waste (S3). In both soils and flower samples a prevalence of obsolete compounds over chlorpyrifos was observed, and the 6-CB predominated among the homologous groups of PCBs These results highlights the importance of soils as sink of these persistent contaminants, which became available depending on environmental conditions. Results revealed that the land uses and seasonal variations have directly impacted on the levels of agrochemicals, PCBs and PBDEs found in the beehive matrixes. This survey provides novel evidence about the current situation of pollution on honey bee colonies under temperate climates and contributes to the knowledge of this poor studied topic in Argentina.

Keywords: POPs, chlorpyrifos, bees, bee bread, honey, land use.

1. Introduction

Honey bees (*Apis mellifera*) are essential insects in preserving ecosystems and human welfare by both pollination of wild plants and increasing the productivity of crops (Klein et al., 2006). They are also considered of great agronomic, environmental and economic importance due not only to pollination buy also to the generation of high value commercial products like wax, pollen, propolis, royal jelly and mainly honey (Gallai et al., 2009; Brown and Paxton, 2009). In order to store enough amounts of these beekeeping products, honey bees fly several kilometers (proximally 3 km; Frisch, 1965) from the colony and inform nestmates of the location of sources of nectar, pollen and resins (Grüter and Farina, 2009). Unfortunately, due to honey bees share space with agricultural settings, there is a greater probability of transporting agrochemicals like pesticides to the colony (Mitchell et al., 2017; Calatayud-Vernich, 2016).

In recent years, a continuous increase in losses of honey bee colonies has been reported because of a variety of causes, including management practices, *Varroa* mite infestation, stress, pathogens, pesticide exposure, and climate change (Mullin et al., 2010; Aldea and Rodriguez, 2014; Nuemann and Carreck, 2010). Particularly, in Argentina between 30-35% of honey bee colonies are being lost every year (Maggi et al., 2013; SENASA, 2015).

Honey bees are exposed to perturbations occurring in the surroundings of crop fields where they habit. In this sense, the colonies are considered good biomonitors and can serve to understand the dynamics of environmental pollutants in the hives (Malhat et al., 2015).

Persistent Organic Pollutants (POPs) are chemicals of global concern due to their persistence in the environment, volatility, ubiquity, lipophilicity, ability to bioaccumulate in different matrixes and biomagnify in ecosystems; as well as, chronic toxicity (UNEP, 2011). POPs include relevant compounds such as organochlorine pesticides (OCPs), polychlorinated biphenyls (PCBs) and

polybrominated diphenyl ethers (PBDEs). All these compounds are forbidden at worldwide level and regulated by the Stockholm Convention (UNEP, 2011).

Current use pesticides (CUPs), such as organophosphates, are compounds more water soluble, less persistent, and less bioaccumulative than OCPs. However, they are used extensively, representing more than 30 % of the total consumption of insecticides worldwide (ISTAT, 2010). For instance, in Argentina, the consumption of pesticides increased by 300% during the last 15 years (CASAFE, 2012) being chlorpyrifos and cypermethrin the most widely used pesticides (Mugni et al., 2010).

The occurrence of pesticide in beehive matrixes have been reported worldwide (Ghini et al., 2004; Chauzat et al., 2011; Mitchell et al., 2017), and some studies have shown that the level of contamination of hives is closely related to the surrounding environment (García-Chao et al., 2010; Mullin et al., 2010; Chauzat et al., 2011; Panseri et al., 2014; Malhat et al., 2015).

Argentina is a country characterized by agricultural production with a primary economy based on soybean, corn, wheat, sunflower, and others (CASAFE, 2011). Organochlorine pesticides were widely used in Argentina until their worldwide prohibition, most of them in the 90´, due to their known adverse effects on health human and the environment (Lepori et al., 2013).

Land use changes result in improper use of agrochemicals and industrial contaminants exposure that can be problematic to honey bees at low concentrations (Mullin et al., 2010). To our knowledge, there is no survey of the extent of POPs and chlorpyrifos accumulation in managed honey beehives from the southeast region of Buenos Aires Province, Argentina. Moreover, there is very scarce information about how legacy pesticide and industrial contaminant residues can be related to foraging landscape surrounding the colony and affect the different matrixes of beehives. The aim of this study was to evaluate the dynamics of persistent organic pollutants (OCPs, PCBs and PBDEs) and chlorpyrifos in matrixes of honey beehive, including bee bread, honey and honey

bees from managed colonies of *Apis mellifera*, and the surrounding environment (soils and flowers), considering spatial and temporal variations.

2. Materials and Methods

2.1 Study area and sampling

Sampling sites were characterized by different land uses: -Site 1 (S1, 37°56'0.69" S; 57°40'40.53" O): farm settled inside a fruit-horticultural belt; -Site 2 (S2, 37°36'34.7" S; 58°01'07.1" W): agricultural field dedicated to extensive soybean production and, -Site 3 (S3, 38°05'20.8"; S 57°37'16.8" W): field adjacent to urban disposal waste (Fig. 1).

Samples of bee bread, honey, worker bees from colonies, in hives and, flowers and surface soil from the bordering of the apiaries were collected in August (Period 1) and November 2016 (Period 2), during pre-application and application periods of chlorpyrifos, and also coincident with relatively low temperatures (3-12°C) and higher temperatures (18-26°C), respectively.

At each apiary, three randomly hives were selected and three (N=3) pools of each matrix were sampled. Bee bread and honey samples were collected in aluminum containers. Worker bee samples were taken from inside the colony using a glass jar. Surface soil samples (N=3) were collected for each site using a steel spoon. Flowers (N=3) were directly taken from plants located in the surrounding areas of the apiary. All samples were transported to the laboratory and stored at -20 °C until analysis.

2.2. Analytical methods

2.2.1. Reagents and standard materials

Dichloromethane and n-hexane (pesticide grade), anhydrous sodium sulfate and silica gel (Merck Inc.) were used. Standard solutions of organochlorine pesticides and PCB-103 (internal standard) were purchased from Absolute Standards and Ultra Scientific, respectively. In the case of PCBs standard solution and PBDEs mixture were obtained from AccuStandard (''Bromodiphenyl Ethers-Lake Michigan Study'' New Haven, CT, USA).

2.2.2. Extraction and clean-up

Extraction of contaminants from honey bees, pollen, soil and flowers were carried out according to Metcalfe and Metcalfe (1997), with modifications of Miglioranza et al., (2003). Briefly, subsamples (*n*=3 for each matrix) of bee bread (2 g), whole body of honey bees (2.5 g, approximately 15 organisms), flowers (3 g) and soil (5 g) were homogenized (mashed) with anhydrous sodium sulfate and spiked with 10 µl of PCB #103 as internal standard. Subsamples were Soxhlet extracted (6 h) with a mixture of hexane-dichloromethane (50:50). Lipids were removed from the extracts by gel permeation chromatography using Bio Beads S-X3 (200–400 mesh) (Bio-Rads Laboratory, Hercules, California). The pollutant fraction was purified by column chromatography with activated silica gel (200 °C for 24 h). Extracts were concentrated to 1 mL and kept at -20 °C prior to gas chromatography analysis.

Honey samples (2 g) were extracted with 20 ml of a mixture of water-acetone (50:50), and spiked with 10 µl of PCB #103 as internal standard. Then, 5 ml of dichloromethane were added and was shaken for 1 h. The mixture was centrifuged at 3000 rpm for 10 min at 10 °C. Then, the solvent phase was transferred to another falcon tube, and other 5 ml of dichloromethane was added and the extraction was repeated. The extract was concentrated to a final volume of 1 mL. The cleanup

was performed by silica gel column, and then the extracts were concentrated to 1 mL and kept in sealed vials at -20 °C prior to GC analyses.

2.2.3. Gas chromatographic analyses

The analyses of OCPs (α-, β-, γ- and δ-HCH, α- and β-endosulfan, endosulfan sulfate, *p,p*'-DDT, *p,p*'-DDE, *p,p*'-DDD, α- and γ-chlordane, aldrin, dieldrin, endrin, heptachlor, heptachlor epoxide), chlorpyrifos, PCBs (IUPAC #8,18, 28, 31, 44, 49, 52, 56, 60, 66, 95, 97, 99, 101, 105, 110, 118, 123, 128, 132, 138, 141, 149, 153, 156, 158, 170, 174, 177, 180, 183, 187, 194, 195, 199, 203, 206 and 209), and PBDEs (#28, 47, 66, 85, 99, 100, 138, 153, 154) were carried out using a Shimadzu gas chromatograph GC-17A with an electron capture detector (ECD), equipped with a fused-silica SPB-5 capillary column (30 m, 0.25 mm i. d., 0.25 um film thickness, Supelco, Bellefonte, PA, USA). One microliter was injected in splitless mode at 275 °C and the ECD was set at 290 °C. The oven temperature program was: 100 °C held for 1 min, followed by an increase of 5 °C/min to 150 °C, held for 1 min, increase 1.5 °C/min to 240 °C, and then 10 °C/min to 300 °C, held for 10 min. Ultra-high purity helium was used as carrier gas (1.5 mL/min) and nitrogen as make-up gas (Miglioranza et al., 2003).

2.2.4. Quality Assurance and Quality Control (QA and QC)

Laboratory quality assurance samples were used to estimate the quality of the analytical data. Laboratory quality control included laboratory blanks and surrogate recovery spikes. Results of laboratory blanks indicate that samples were not contaminated due to processing in the laboratory. Method detection limits (MDLs) were calculated as three times the standard deviation of the blanks plus three standard deviations. Instrumental detection limits, calculated according to Keith et al., (1983), ranged between 0.03 to 0.05 $ngml^{-1}$ for HCHs, 0.19 $ngml^{-1}$ for chlorpyrifos, and

between 0.08 to 0.33 $ngml^{-1}$ for the remaining compounds. Final MDLs ranged between 0.015-0.165 ng per sample (honey bee, bee bread and honey); and between 0.006-0.066 ng for soil and flower samples. Recoveries, calculated by a spiking matrix and surrogate recovery (PCB #103) were greater than 90%.

2.3. Statistical analyses

The data reported in this work are expressed as nanogram per gram on a dry weight basis for soils, and nanogram per gram on a wet weight basis for honey bees, pollen, honey and flowers.

Non-parametric analyses of the Kruskal-Wallis test were calculated when possible to compare POPs concentrations. Data were processed with statistical software InfoStat version 2013 (Di Rienzo et al. 2013). Probability values less than 0.05 ($p < 0.05$) were considered as statistically significant.

3. Results and discussion

3.1 Honey bees

The total mean concentrations of pesticides, PCBs and PBDEs in honey bee samples are summarized in the Fig. 2 and Table S1 and S2 of the Supporting Information. The total pollutant concentrations varied between <dl-80.04 ng/g ww. The highest total pesticide concentration was registered in the soybean field (S2, 20.21 ng/g), dedicated to extensive agricultural practices; and in the field adjacent to the land of final disposal of waste (S3, 19.79 ng/g) in Period 2, followed by the Santa Paula farm (S1), S2 and S3, from Period 1, which did not show significant differences among sites (range: 10.37-12.29 ng/g; $H= 0.36$, $p > 0.05$). Since higher contaminant concentrations were found during Period 2, it could be attributed to higher temperatures (summer) that enhances

volatilization and remotion of these compounds from areas where they were applied and accumulated. It is important to highlight that most of pesticides found are forbidden, at exception of chlorpyrifos that is currently used (Jergentz et al., 2005; Álvarez et al., 2013). Other studies have found similar results in relation to endosulfans and DDTs, showing the important role of temperatures in these processes (Silva Barni 2018; Agrell et al., 1999). A distribution pattern PCBs > pesticides was observed in most sampling sites at both periods (pre-application and application of pesticides), at exception of S2 at Period 1.

Total DDTs concentration in honey bee samples were in the range of <dl-11.25 ng/g (overall sites and periods combined, Fig. 3), showing the highest levels among the target analytes. DDT is readily transformed to the stable and toxic metabolites *p,p´*-DDE and *p,p´*-DDD under aerobic and anaerobic conditions, respectively (Malhat et al., 2015). The *p,p´*-DDD was detected in the honey bee samples in both periods and in all sampling sites. Despite of DDT has been totally banned in Argentina in 1998 (González García, 2003), DDTs residues can still be present in the environment as a consequence of its high persistence as well as fresh inputs through the use of the acaricide Dicofol (El-Zoghby et al., 2000; Ahmed et al., 2001). It is known that DDTs are generated during manufacturing Dicofol, becoming impurities of the acaricide (Qiu et al., 2005). In addition, DDT has been detected in antifouling paints (Yu et al., 2011a, 2011b). The levels of DDT metabolites found in this study (range 3.30-11.25 ng/g) were lower than those found to be inhibitory for the enzime ATPase potassium and calcium channels (Koch et al., 1968).

Endosulfans were detected in the honey bee samples in both periods and in all sampling sites (Fig. 3). It is a broad-spectrum insecticide used on soybean, cotton, tomato, and other crops. The composition of endosulfan isomers can reveal their historical or recent inputs into the environment. The technical mixture comprises 70:30 of the mixture α–/β- isomers, being the main

biological metabolite, the endosulfan sulfate. The honey bee samples from S1 and S2 in Period 1 showed the ratios α–/β-endosulfan > 10, which indicate relatively fresh inputs of technical endosulfan. Nevertheless, endosulfan has been forbidden on Argentina since 2013, so the levels found in this study could suggest use of remaining stocks, or also the volatilization from other areas or regions. Besides, the concentrations of α- and β–endosulfan observed in this study (with ranges of <dl-3.48 and 0.18-1.60 ng/g, respectively) were within those found by Mullin et al., (2010), for honeybee samples from USA (1.3 to 6.1 and 1.4-2.4 ng/g, respectively for each isomer). On the other hand, concentrations of these pesticides were found in watersheds close to the sampling area, such as in the Quequén Grande River watershed, where endosulfan was the main OCP found in both soil and water samples (Gonzalez et al., 2013; Silva-Barni et al., 2019).

HCHs residues were found in all honey bee samples except in S1 in Period 2, with a range of <dl-2.90 ng/g (Fig. 3). The β-HCH was the isomer mainly detected, found in Period 1 with a range of 0.17-2.90 ng/g, while γ-HCH was found in Period 2 in S2 y S3, with a range of 0.02-0.12 ng/g. The technical mixture was prohibited in Argentina in 1998 (Lolster and Krapovickas, 1999), it primarily consists of isomers α-HCH (55-89%, w/w), β-HCH (5-14%), γ-HCH (8-15%), and δ-HCH (2-16%) while lindane mainly contains γ-HCH (>98%) (Li et al., 2006). Among the isomers, β-HCH is the most persistent, less volatile, and tends to be more bioaccumulated than other HCH isomers (Wang et al., 2010). Thus, this isomer would reflect historical pollution of technical HCHs in the study area.

Heptachlor was prohibited in 1998 in Argentina (Lolster and Krapovickas, 1999), but until then, it was extensively used on potato crops (Miglioranza et al., 2003). Heptachlor epoxide is the primary degradation product, and it is more likely to be found in the environment than heptachlor (Bidleman et al., 1998). Moreover, concentrations of this metabolite were found in soils of

Quequén Grande River watershed (Silva-Barni et al., 2018; Gonzalez et al., 2012), reflecting the historical usage of heptachlor in the vicinity of the study area. This compound was observed in Period 1 in all sampling sites, with a range of 0.34-1.16 ng/g; while heptachlor epoxide was predominantly found in Period 2 with concentrations lower than 0.4 ng/g (Fig. 3). On the other hand, the chlordane levels found in the honey bee samples were below 1 ng/g, most likely as a consequence of past use in the studied sites.

Dieldrin was used in Argentina to control soil agricultural pests before its ban in 1980. Dieldrin is also generated in the environment as a metabolite of aldrin, being more resistant to biotransformation and abiotic degradation than aldrin. In this survey, dieldrin was mainly found in S3 with concentrations of 0.12 and 0.44 ng/g, for pre-application and applications periods of pesticides, respectively (Fig. 3). Additionally, endrin is an isomer of dieldrin and may be metabolized by animals. Endrin was primarily used as an insecticide and has been also employed as a rodenticide, avicide, and on some fish farms had been used as a piscicide. In this survey, endrin was found in S2 in Period 2, and in S3 in Period 1. There was no evidence of a relationship between endrin concentrations with the periods and/or sampling sites, suggesting a non point source in the area.

Chlorpyrifos was the second most abundant pesticide detected in this study (Fig. 3). The highest concentration was found in S2 in Period 2 (8.88 ng/g), which could related to the current use since it is a field dedicated to extensive soybean cultivation and the period corresponds to pesticide application. Chlorpyrifos, is an organophosphate pesticide used to protect food crops against insects and mites (Solomon et al., 2014). It is considered ubiquitous with a moderate persistence in the environment because of its high use and potential to volatilize and disperse by air (Davie-Martin et al., 2012). The levels found in this study were higher than those reported in honey bees

from New Zealand, with a range of 0.30-2.83 ng/g (Urlacher et al., 2016), and similar to those found by Mullin et al., (2010) in North America, which was 30-40 ng/g. Chlorpyrifos has also been detected in bees at higher concentrations in Egypt (31 ng/g; Al-Naggar et al., 2015), Poland (3.5 ng/g; Kiljanek et al., 2017) and France (1.72-180.20 ng/g; Lambert et al., 2013). Naggar et al., (2015) found that gene expressions of hymenoptaecin, as a measure of stress exposure, were greater in honey bees with food containing 7.7 ng/g of chlorpyrifos. This value is within the range of concentrations detected in the present study; therefore, the honey bees analyzed could be undergoing modifications in the expression of such gene, and consequently.

Total concentrations of PCBs in honey bee samples ranged between 7.68-47.74 ng/g (Fig. 2, Table S2). Among PCBs groups, there was a clear predominance of 3-CB in all sampling sites and periods, followed by 4-CB (Fig. 4). It is known that light chlorinated biphenyls are more volatile, being able to undergo long-range transport and dominate the pattern in the air, so they are easily transported by atmospheric processes (Negoita et al., 2003). The highest concentrations of PCBs found in S3 in both periods, could be due to the activities developed in this study area. The municipal waste disposal of Mar del Plata city, with a surface of 102 hectares, constitutes one of the main open dumping sites in the zone, which receives 1150 ton per day of municipal wastes (MGP, 2013). Therefore, municipal solid waste would be a possible source of industrial pollutants (Ham et al., 2008; Sakai et al., 1998). In addition, the predominant winds from west side could lead to enhance the atmospheric transport of contaminants to urban areas. On the other hand, in S2 during Period 2, high concentrations of 3-CB were also found, it is presumably due to higher temperatures lead to more evaporation. Therefore, the higher proportion of lighter congeners in the honey bee samples could be attributed to re-evaporation from secondary sources as well as atmospheric transport from urban sites. Otherwise, in S1, 3-CB was the predominant PCB group;

however a relationship between sampling sites and periods was not observed, suggesting background levels in the area as a result of the past use of these compounds.

Total concentrations of PBDEs in honey bee samples ranged between <dl-80.04 ng/g (Fig. 2, Table S2). The congeners #28, 47 and 99 were the only detected, and the highest concentration corresponded to congener #47, found in S2 and S3 during Period 2 (41.59 and 79.51 ng/g, respectively). It is known that the occurrence of #47 as the main congener is often associated to atmospheric transport from surrounding areas. Moreover, the lack of congeners #100 and #99 in most of the sites supports this statement (de Wit, 2002). Moreover, other reports have informed high PBDEs concentrations associated to urban garbage dump, plastic and electronical disposal (Miglioranza et al., 2013a, Commedatore et al., 2018). Therefore, the higher concentration of congener #47 in the honey bee samples from S3, could be attributed to the proximity of municipal waste disposal of Mar del Plata city.

To our knowledge there are not surveys about PCBs and PBDEs residues in honey bee samples obtained directly from the inner of the hive, both in Argentina and at worldwide level.

3.2. Bee bread

Successive pollen loads (as pellets) in the cells, form a stratified column, named bee bread, and then the nurse bees convert the nutrients of bee bread into jelly (Crailsheim, 1992). Therefore, the concentrations of pesticides present in bee bread are much closely to those found in the environment (Sponsler and Johnson, 2016). So, if the pollen is contaminated by pesticides these residues could affect almost all the colony.

The total mean concentrations of pesticides, PCBs and PBDEs in bee bread samples are summarized in the Fig. 2 and in the Table S1 and S2. A general pattern, pesticides > PCBs > PBDEs

was observed, and a relationship pesticides/PCBs+PBDEs > 1 was found in the most sampling sites in both sampling periods, at exception of Period 2 from S2 and S3. The total pesticide concentrations varied between 1.88-8.96 ng/g, ww, and were higher in Period 2 than Period 1 in almost all sampling sites. As was observed for honey bee samples, the higher concentrations found during pesticides application period could be associated with the higher temperatures (summer) that enhances pesticide availability, leading to higher volatilization. In the case of chlorpyrifos it is coincident with its application period. Despite there was not a significant difference among concentrations, a clear tendency was observed.

Endosulfans were the most abundant between organochlorine pesticide in the bee bread samples, and were detected in all samples with a range of <dl-4.66 ng/g, with the exception of S1 during Period 2 (Fig. 3). It was observed a relationship α-/β- isomers ≥1 in samples from S1 during Period 1 and from S3 in both sampling periods. These results suggest a possible use of the technical endosulfan mixture in the surrounding areas despite its forbidden use. The isomers α- and β-endosulfan were detected in a range of <dl-4.66 and <dl-0.42 ng/g, respectively, while endosulfan sulfate was only detected in S2 in Period 1 (0.26 ng/g). These values were lower to those found by Mullin et al., (2010) in USA.

DDTs residues were found in all bee bread samples except in S1 in Period 1, with a range of <dl-1.90 ng/g (Fig. 3). Residues of *p,p´*-DDD were only found in Period 2 in all the sampling sites in the range of 1.70-1.90 ng/g as a result of the past use of DDT. As was previously described by Eggen and Majcherczyk (2006), under natural conditions, the low temperature lead to a slow degradation of DDTs, and it could justify the levels below detection limit of metabolites during Period 1 (low temperatures). The levels of this compound found by Mullin et al., (2010) in pollen samples from USA were much higher than those found in this study, with a range of 11.8-13.4

ng/g. Despite the low values found for DDT group, it highlights the importance of legacy pesticides in the environment, which constitute a continue concern to the ecosystems.

Chlorpyrifos was the pesticide most predominant in the bee bread samples. It was found in both periods and all sampling sites (range: 0.52-5.69 ng/g, Fig. 3). The site S1 showed the highest concentration of this pesticide, mainly in Period 2, and it is in line with its current use on many crops in the area. However, non-significant differences were found ($p > 0.05$). The chlorpyrifos levels found in this work were much lower than those found in bee bread from agriculture areas from China (41.4 ng/g; Tong et al., 2018), while were similar to those found in 49 apiaries from Virginia, USA (4.27 ng/g; Fulton et al., 2019). Moreover, Tong et al., (2018) have also reported concentrations of chlorpyrifos in pollen. In this regards, they found similar mean levels of this pesticide in both matrixes (pollen and bee bread), suggesting that those pesticides that reach the flowers can directly impacts on beehives.

Total concentrations of PCBs in bee bread samples ranged between 0.44-12.80 ng/g (Fig. 2, Table S2). These compounds were found in all bee bread samples with the highest concentrations in S3 followed by S2 and S1 (Kruskall Wallis H= 5.96, $p < 0.05$). In all sampling sites a similar trend was observed, with the 3-CB as the group with the highest concentration with a range of <dl-10.11 ng/g (Fig. 4). As was observed in the honey bee samples, the 3-CB predominated in all sampling sites and periods, and the highest concentration corresponded to S3 in Period 2, with mean value of 10.11 ng/g, being the #18 the main congener found among the homologue groups. The site S3 is very close to the municipal waste disposal area and therefore the results would be related to the land use. The 6-CB congeners were also observed in almost all samples, except S2 in Period 1, and S3 in Period 1, where 3-CB was the predominant PCB group (#28+31).

Total concentrations of PBDEs ranged between <dl-0.69 ng/g (Fig. 2, Table S2). These compounds were only detected in Period 2 in all sampling sites, and it was observed a general pattern S3 > S2 ≥ S1. This fact is also related to the clear impact of land use. The highest concentration corresponded to congener #47, with mean value of 0.69 ng/g. The dumping areas, atmospheric transport, as well as the close urban and industrial activities, would contribute to PBDEs levels in S3. In this way, the municipal waste disposal could be a secondary source of PBDEs to the environment.

Pollen is used by nurse bees to produce jelly to feed larvae, the queen, drones and older workers (Crailsheim et al., 1992; Crailsheim, 1992) and if the pollen is contaminated, these residues could affect almost all of member from the colony, since it is known that the pollen influences the physiological metabolism, immunity (Alaux et al., 2010), tolerance to virus and pathogens (Rinderer et al., 1974) and reducing the sensitivity to pesticides (Wahl and Ulm, 1983).

Several biological and physicochemical processes occur when contaminant residues enter the hive (Tremolada et al., 2003). The integration of the active ingredients in the products of the hive can be generated by bees and air, acting as distribution pathways. In addition, the redistribution among the different compartments is determined by the physic-chemical properties of the compound and the characteristics of the hive matrices (Tremolada et al., 2003). Air is an important compartment inside the hive, and it allows a rapid internal redistribution between the other compartments. Among these compartments, exchange processes and partitioning exist, such as volatilization and deposition between air and solid phases and partitioning between solid and liquid phases contact, like beeswax and bee bread (Tremolada et al., 2003). Therefore, those pesticide residues in beeswax or other hive matrix could be transferred to bee bread samples. Thus, the compounds found in this survey could come directly from contaminated environment or

from other hive matrix. According to Niell et al., (2017), residues in beeswax represent an excretion product of the bees, a way to eliminate the toxic compounds from their bodies. In this sense, the wax combs could also be a potential secondary source of pesticide and industrial compound residues.

3.3. Honey

The contamination of honey by pesticides may occur through direct contamination from beekeeping practices as well as indirect contamination from environmental sources through air, water, plants and soil and then be transported into the beehive by the bees (Anderson and Wojtas, 1986; Kujawski et al., 2012; Panseri et al., 2014). The following distribution pattern pesticides > PCBs > PBDEs was observed in all sampling sites in both study periods. The total pollutant concentrations ranged between <dl-11.47 ng/g, ww, and the highest levels were found in Period 1 in all sites (S1: 10.23 ng/g; S2: 10.29 ng/g; S3: 11.47 ng/g) (Fig. 2, Table S1 and S2). This fact could be attributed to previous applications of pesticides to the time of the sampling. Residues of legacy pollutants, presumably from past agricultural application and other uses, are still in many environmental compartments and continue to cycle through various routes, such as atmospheric transport and runoff (Blasco et al., 2004).

DDTs residues were the most abundant in the honey samples and they were found in all samples with a range of <dl-7.66 ng/g (Fig. 3). Little is known about the metabolite degradation of DDTs in honey (Panseri et al., 2014), but the levels of *p,p´*-DDE and *p,p´*-DDD in honey samples found in this study were lower than those reported in Egypt (19 ng/g and 8 ng/g, respectively; Malhat et al., 2015), except to *p,p´*-DDD levels from S3 in Period 1 which showed similar values (7.66 ng/g; Table 1). In addition, Panseri et al., (2014b) reported *p,p´*-DDE values (5.4 ng/g) higher than those found

in this study (2.44 ng/g) in honey samples collected from different areas of Italy dedicated to intensive orchards. In this survey, there was a relationship DDE+DDD/DDT>>>1, which indicates no fresh input of DDT in the study area. The metabolites could have the source directly from pollen and also could have been metabolized in the hive from the parental compound, DDT.

HCHs residues were found in all the honey samples (range: <dl-2.14 ng/g, Fig. 3). β-HCH was detected in almost all sampling sites in the range <dl-2.14 ng/g, with the highest values in S1 and S2. These values were lower than those found by Blasco et al., (2004) for honey samples from the central zones of Portugal and Spain (17.5 and 6.56 ng/g, respectively). In addition, γ-HCH was also detected in all the sites and sampling periods, and the values found in this survey was lower than those found by Malhat et al., (2015) in samples from Egypt (mean value: 9.40 ng/g).

Endosulfans were found in all honey samples (range: <dl-1.48 ng/g, Fig. 3). The α-endosulfan residues, showed values slightly below those reported by Choudhary and Sharma (2008) in samples from India (1.55 ng/g). Regarding β-endosulfan, the values varied from <dl to 0.48 ng/g, and also were below to the values from Indian samples (2.27 ng/g; Choudhary and Sharma, 2008), and from Italy, which were honey samples from an intensive farming 20.6 ng/g (Chiesa et al., 2018). On the other hand, endosulfan sulfate concentrations found in S1 and S2 during Period 1 (1.06 and 1.12 ng/g, respectively) were lower than those reported by Medici et al., (2019) in apiaries located in the main agro-industrial areas from Argentina (11.30 ng/g).

Chlorpyrifos residues were detected in all honey samples with a range of 0.76-3.89 ng/g (Fig. 3). The highest concentration was found in the site S1 in Period 2, which could be explained by the intensive use of this pesticide. In addition, the levels of chlorpyrifos found in this study, were lower than those reported in intensive orchards from Italy (9.4 ng/g; Panseri et al., 2014). However, other study carried out in Greece, found levels of chlorpyrifos in honey samples from different

agricultural areas, in the range of 0.70-0.89 ng/g (Balayiannis and Balayiannis, 2008), while in Uruguay, chlorpyrifos was one of the most detected compounds and with the highest concentrations ranging from 30 to 80 ng/g (Pareja et al., 2011), exceeding widely the concentrations of this study. Thus, the presence of this insecticide could be explained by the stability of its molecule to hydrolytic, oxidative, and metabolic processes, its low volatility, and its excessive use by farmers (Davie-Martin et al., 2012).

The values of pesticides found in the honey samples were below the maximum limits established by the Ministry of Agriculture, Livestock, Fisheries and Food from Argentina (SAGPyA, Res. 125/98); however, it has been reported that the presence of these contaminants in beehaves could have deleterious synergistic effect on the acaricides used in beekeeping (Tomé et al., 2017).

In regard to PCBs, the highest concentrations were registered in Period 1 at all sampling sites, with the following pattern S1 > S2 > S3 (Fig. 2, Table S2). The concentrations of PCB groups are shown in the Figure 3. In S1, the 6-CB and 5-CB predominated over the others homologue groups in Period 1. The congeners #153+132 and #101 showed the highest concentrations (2.29 and 1.00 ng/g, respectively); while in Period 2, the 4-CB and 6-CB were only detected; moreover the congener #66 showed the highest concentration (0.14 ng/g) among other congeners. By other hand, in S2, at Period 1, the congener #209 showed the highest levels followed by 6-CB and 8-CB groups. In Period 2, however, predominated the lower chlorinated congeners at expenses of congener #52 as the only (0.79 ng/g). In S3, the light congeners #28+31 showed the highest levels in Period 1 (3.40 ng/g). It is important to note that as was observed in the other matrixes, the occurrence of municipal waste disposal close to sampling site is an important factor to influence on contaminant levels in hives and the surrounding ecosystem. Regarding PBDEs, only congener

#28 was detected in Period 1 with at mean concentrations of 0.99 and 0.96 ng/g, for S1 and S2, respectively (Fig. 2, Table S2).

The levels of these contaminants found in honey could be a consequence of direct air transport from the dumping site, from the nectar and sweet deposits from contaminated plants, which are used to get the honey or also through cross contamination inside the hive.

3.4. Surrounding environment of hives

3.4.1. Soil

Contaminants enter the soil by deposition from air, drift, or in the case of pesticides by washing-off from plant surfaces during rainfall or irrigation, and are adsorbed by organic matter or clay materials (Miglioranza et al., 2003). Thus, soils act as sink or source of contaminants to the environment, being available for biota. Soil samples collected from surrounding areas to the hives showed a similar pattern of pollutants, pesticides > PCBs > PBDEs in all samples. The total contaminant concentrations ranged from <dl to 4.45 ng/g, with the highest values in S3 (4.45 ng/g) followed by S1 (3.34 ng/g) from Period 1, and S2 (3.30 ng/g) from Period 2 (Fig. 5, Table S3 and S4). These levels were lower than other reported for soils from Argentina, such as Rio Negro watershed (1275 ng/g ww), where an intensive apple culture is developed as the main activity in the zone (Miglioranza et al., 2013). Moreover, Lupi et al., 2016, in a close area to the sampling site of this study, with similar agricultural activities, found total pollutant levels in the range to 0.18-0.71 ng/g dw. Therefore, the soil concentrations reported in this study are considered as “clean soils” to worldwide level, and would not represent a concern to the related ecosystems (Department of Soil Protection, 1994).

DDTs residues were most abundant pesticide in the soil samples, with a range between <dl to 2.77 ng/g (Fig. 6), with a predominance of metabolites (*p,p´*-DDE: <dl to 0.40 ng/g, and *p,p´*-DDD

residues: <dl to 2.77 ng/g). This result is in line to the past use of the parental DDT, and also could be a consequence of the current use of Dicofol (impurities of DDT). The half life of DDT in soils is usually higher than 25 years, depending of soil characteristics (Diamond and Owen, 1996), and therefore this matrix play an important role as sink of these kinds of pesticides. Similar results were found in soil samples from an agricultural area, settled close to the study area, with DDT metabolites values of 0.81 ng/g for *p,p´*-DDE and 0.03 ng/g for *p,p´*-DDD (Miglioranza et al., 2003). Other soils from the Rio Negro watershed (Argentina) with intensive agricultural activities, such as fruit production, and massive pesticide use, presented higher levels of DDTs metabolites (188.2 ng/g ww), being *p,p´*-DDE the main metabolite registered (Miglioranza et al., 2013). The *p,p´*-DDE levels found in this study are in line with those reported by Rissato et al., (2006) in soil samples from Brazil (2.05-8.8 ng/g).

HCHs residues were found in all soil samples (range: <dl-0.49 ng/g, Fig. 6). Among HCH isomers, the isomer β-HCH was detected at higher concentrations in all sampling sites and both periods, except in S1, at Period 1. These values were higher than those reported in Los Padres lagoon watershed (0.20 ng/g; Miglioranza et al., 2003), and were also higher than levels found in Brazil with a range of <0.05-0.34 ng/g (Rissato et al., 2006). The prevalence of β-HCH in soils would reflect a legacy use of these pesticides in the region and due to its high persistence β-isomer is usually found adsorbed to soil surfaces.

In S2, the highest levels of chlorpyrifos were found in Period 2, while non-significant differences were found in S3 for both periods (0.26-0.40 ng/g; $H= 2.33$; $p > 0.05$). It is important to note that chlorpyrifos represents the main insecticide used in Argentina under soybean cultures, the main activity developed in S2. Therefore the surrounding environment, including biotic and abiotic compartments would be exposed to this pesticide (CASAFE, 2012; Alvarez et al., 2013).

Total concentrations of PCBs in soil samples ranged between 0.60-2.46 ng/g (Fig. 5, Table S4). The highest PCBs concentrations were found in S2 (2.46 ng/g) and S3 (2.23 ng/g) at Period 2. According to land use, S3 showed similar concentrations between both sampling periods, compared to the other sampling sites. There was a predominance of 6-CB in both periods, followed by 3-CB and 4-CB (Fig. 6). Hexa-CB congeners were found in agriculture areas from Brazil with lower values (<0.02 to 0.23 ng/g) than those found in the present study; and in the case of 3-CB and 4-CB concentrations, they were higher than those reported by by Rissato et al., (2006), with a range of 0.03-0.25 ng/g for both groups of contaminants. The #153 was the only detected congener from 6-CB group with a mean concentration of 1.17 and 1.65 ng/g for Periods 1 and 2, respectively. This compound is the major constituent of both Aroclor 1254 and Aroclor 1260, and it is characterized by being readily bioaccumulated and resistant to metabolic and microbial breakdown (Hawker and Connell, 1988; Sundstrom et al., 1976).

On the other hand, total concentrations of PBDEs in soil samples ranged between <dl to 0.78 ng/g (Fig. 5, Table S4). The #28 was the unique congener found in S1 an S2, while in S3 there was more variability of congeners (#28, 100 and 153), despite they were only found during Period 1. The highest concentrations were in S2 during Period 1, where the congener #28 showed 0.52 ng/g. Thus, the urban and industrial activities in addition to atmospheric transport, could contribute to PBDEs levels in soil samples. The values found in this work were similar to those found in soils from the Río Negro basin, where residues were detected at levels below 1 ng/g dw (Miglioranza et al., 2013). On the other hand, PBDEs values reported in this study were much lower than those reported for soils from other countries: Indonesia (0.11-255 ng/g dw), Cambodia (0.55-91 ng/g dw), Vietnam (1.2-429 ng/g) and India (0.82-19 ng/g dw; Eguchi et al., 2009); but were similar to those found in Swedish agricultural soils (0.23 ng/g dw) by Matscheko et al., (2002).

3.4.2. Flowers

The concentrations of pollutant residues detected in flower samples are found in Tables S3 and S4. The general contaminant pattern was pesticides > PCBs > PBDEs, and the levels of total pollutant ranged from, <ld to 15.52 ng/g (Fig. 5). Samples from Period 2 registered higher pesticide values in all sampling sites, when available (Fig. 4). The total pollutants levels found in flowers were higher than soil samples. It could be related to the fact of translocation from the soil to the flower, or also through air deposition (wet and dust) above flowers (Krupke et al., 2012).

In S1, endrin ketone showed the highest concentrations in both periods (3.40 and 3.49 ng/g, respectively), while in S2, only during Period 2 (4.70 ng/g). In S3, the highest concentration corresponded to β-HCH, found during Period 1 (1.16 ng/g). Chlorpyrifos and endosulfans presented the highest values in S2 among all sampling sites (4.15 and 2.04 ng/g, respectively). Particularly, since endosulfan sulfate was not found in any samples, it could indicate a recent use of the technical mixture in the area, despite its forbidden use. The occurrence of chlorpyrifos in flowers mainly during application period it is directly related to its current use. The chlorpyrifos is usually applied on corn, soybean, peach and kiwi, which are developed in the sampling sites (Bedmar et al., 2015).

Regarding to pesticides, the HCH residues were the most abundant compound in flower samples, with a range of <dl-1.40 ng/g. The highest concentrations were found in S1 and S2 during Period 2. In addition, drins residues were found in all flower samples, with the metabolite endrin ketone being the main pesticide of the group.

It is important to note that pesticides found in flowers could be a potential source of contaminants to the other matrixes in the hive, such as by the nectar during honey production or by contact with

the wax. Therefore the levels of pesticide in flowers constitute a way for contaminants enter to the hive.

Regarding to PCBs, the total concentrations ranged between <dl to 10.78 ng/g (Fig. 7). The 6-CB was the homologous group with the highest levels in almost all sampling sites and periods with a range of mean concentrations of <dl-8.06 ng/g, being #128 and #138 the main congeners found. The #153 congener, was the 6-CB mostly detected in flower samples, similar to the results found in soil samples, as a consequence of its recalcitrant characteristics. This compound showed the highest values during Period 1 in S1 (2.89 ng/g) and S3 (2.99 ng/g). The rest of the congeners were below to 1.5 ng/g.

In the case of PBDEs, the total concentrations were in the range of <dl-1.91 ng/g. The congener #47 was the main compound detected in almost all sampling sites and periods. The highest levels of this congener are in agreement with other literature, because it is the most ubiquitous PBDE as a result of atmospheric transport (de Wit, 2002). Other studies in biotic and abiotic matrixes have attributed the PBDEs pattern with predominance of #47, 99 and 100 to the use of the Penta-BDE commercial mixture (Peng et al., 2018). Curently Penta- and Octa-BDE mixtures have been restricted in some regions of the world, and both mixtures are regulated by the Stockholm Convention since 2008, due to their ubiquitous presence, bioaccumulation, and toxicity.

4. Conclusions

Honey bee, bee bread, honey, soil and flower samples analyzed in the present study revealed the occurrence of OCPs, PCBs and PBDEs residues. In spite of these compounds are forbidden at worldwide level, their residues still appear in both biotic and abiotic matrixes being a concern to beehives sustainability. Chlorpyrifos, a current use pesticide, was detected in all samples from the

entire beehive and also the surrounding environment as a consequence of its heavy use in extensive and intensive agricultural practices. The impact of land uses on contaminant residues in different beehive matrixes was relevant and significant in some scenery, mainly for PCBs and chlorpyrifos, highlighting the municipal disposal waste and agriculture as the main forces responsible to these outcomes.

Among honey beehive matrixes, honey bee samples showed the highest pollutant levels, with a predominance of the industrial contaminants over pesticides. These organisms are more exposed to environmental pollutants than other beehive matrixes; therefore it is recommended to plan monitoring program in order to protect the pollination services in the area.

In the bee bread samples the absence of a common distribution pattern in relation to land use and sampling periods, would be a consequence of a great diversity of pollen sources and their processing and packaging by the bees inside the hive cells.

A predominance of pesticides in honey samples was observed ~~in collected~~ at all the sampling sites and periods, at expenses of both, OCPs and chlorpyrifos. This fact, argues strongly for urgent changes in regulatory policies regarding pesticide monitoring procedures not only for exportation but also for inner commerce.

The highest levels of total contaminants observed in flower samples during the application period of pesticides in agricultural sites, would respond to the common practices regarding to chlorpyrifos use, and the high temperatures that enhance contaminant atmospheric transport.

In both, soils and flower samples a prevalence of obsolete compounds over chlorpyrifos was observed, and the 6-CB predominated among the homologous groups of PCBs. Results revealed that the land uses and seasonal variations have impacted directly the levels of pesticides, PCBs and PBDEs found in the beehive matrixes. Moreover, this survey provides novel evidence about the

current situation of pollution on honey bee colonies under temperate climates and contributes to the knowledge of this poor studied topic in Argentina. Future studies are needed to analyze how these pollutants are affecting bees.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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References

Agrell, C., Okla, L., Larsson, P. and Wania, F. Evidence of latitudinal fractionation of polychlorinated biphenyl congeners along the Baltic Sea region. 1999. Environmental Science and Technology 33, 1149–1156.

Ahmed, T. Loutfy, N., Youssof, Y., El Shiekh, I. and Eissa, I. Residues of chlorinated hydrocarbons, polycyclic hydrocarbons and polychlorinated biphenyls in some marine organisms in Lake Temsah, Suez Canal, Egypt. 2001. Aquat. Ecosyst. Health 4, 165-173.

Al Naggar, Y., Codling, G., Vogt, A., Naiem, E., Mona, M., Seif, A. and Giesy, J. P. Organophosphorus insecticides in honey, pollen and bees *(Apis mellifera L.)* and their potential hazard to bee colonies in Egypt. 2015. *Ecotoxicology and environmental safety*, *114*, 1-8.

Alaux, C., Dantec, C., Parrinello, H. and Le Conte, Y. Nutrigenomics in honey bees: digital gene expression analysis of pollen's nutritive effects on healthy and varroa-parasitized bees. 2011. BMC Genomics 12: 496. doi: 10.1186/1471-2164-12-496. PubMed: 21985689.

Alaux, C., Ducloz, F., Crauser, D., and Le Conte, Y. Diet effects on honeybee immunocompetence. 2010. Biol Lett 6: 562-565. doi:10.1098/rsbl. 2009.0986. PubMed: 20089536.

Aldea, P. and Rodríguez, R. Factors affecting sanitary control of Varroa destructor in Chile. 2014. The IV COLOSS Workshop on "Varroa control strategies" in Bled, Slovenia May 22- 23. Abstract book pp. 32.

Álvarez, M., Du Mortier, C. and Cirelli, A. F. Behavior of insecticide chlorpyrifos on soils and sediments with different organic matter content from Provincia de Buenos Aires, República Argentina. 2013. Water, Air, and Soil Pollut. 224 (3), 1–6.

Álvarez, M., Du Mortier, C., Sokolic, T. and Cirelli, A. F. Studies on the persistence of a commercial formulation of chlorpyrifos on an agricultural soil from Provincia de Buenos Aires, República Argentina. 2013. *Water, Air, & Soil Pollution*, *224*(5), 1571.

Anderson, J. F. and Wojtas, M. A. Honey bees (Hymenoptera: Apidae) contaminated with pesticides and polychlorinated biphenyls. 1986. *Journal of Economic Entomology*, *79*(5), 1200-1205.

Anonymous. Environmental protection, environmental fate. 2009. http://www.al.gov.bc.ca/pesticides/c_2.htm. 28 April 2009

Balayiannis, G. and Balayiannis, P. Bee honey as an environmental bioindicator of pesticides' occurrence in six agricultural areas of Greece. 2008. *Archives of Environmental Contamination and Toxicology*, *55*(3), 462.

Bedmar, F., Gianelli, V. R., Angelini, H. P. and Viglianchino, L. E. Riesgo de contaminación del agua subterránea con plaguicidas en la cuenca del arroyo El Cardalito, Argentina. 2015. Gerencia de Comunicación e Imagen Institucional, DNA SICC, INTA.

Beekman, M. and Ratnieks, F. L. W. Long-range foraging by the honey bee, Apis mellifera L. 2001. Funct Ecol 14:490–496.

Bidleman, T. F., Jantunen, L. M. M., Wiberg, K., Harner, T., Brice, K. A., Su, K., Falconer, R. L., Leone, A. D., Aigner, E. J. and Parkhurst, W. J. Soil as a source of atmospheric heptachlor epoxide. 1998. *Environmental Science & Technology*, *32*(10), 1546-1548.

Blasco, C., Lino, C. M., Picó, Y., Pena, A., Font, G. and Silveira, M. I. N. Determination of organochlorine pesticide residues in honey from the central zone of Portugal and the Valencian community of Spain. 2004. *Journal of chromatography A*, *1049*(1-2), 155-160.

Bortolotti, L.; Montanari, R.; Marcelino, J.; Medrzycki, P.; Maini, S. and Porrini, C. Effects of sub-lethal imidacloprid doses on the homing rate and foraging activity of honey bees. 2003. Bull. Insectol. 56, 63–67.

Brown, M. J. and Paxton, R. J. The conservation of bees: a global perspective. 2009. *Apidologie*, *40*(3), 410-416.

Calatayud-Vernich, P., Calatayud, F., Simó, E. and Picó, Y. Efficiency of QuEChERS approach for determining 52 pesticide residues in honey and honey bees. 2016. *MethodsX*, *3*, 452-458.

Carrillo, M. P., Bovi, T. D. S., Negrão, A. F. and Orsi, R. D. O. Influence of agrochemicals fipronil and imidacloprid on the learning behavior of Apis mellifera L. honey bees. 2013. Acta Sci. Anim. Sci. 35, 431–434.

CASAFE. Mercado Argentino 2011 de Productos Fitosanitarios. Preparado por: Kleffmann group, Argentina. 2011. [on line] http://www.casafe.org/pdf/Informemercadofitosanitarios2011.pdf 3/5/2013.

CASAFE (Cámara de Sanidad Agropecuaria y Fertilizantes, Buenos Aires, Argentina) (2012). Available from: <http://www.casafe.org>. Accessed October 2012.

Cessna, A. J. Pesticides in the environment: Real or Imagined. Agriculture and Agri-food Canada. 2009. Research Centre, Lethbridge, AB. http://www.ssca.ca/conference/conference 2005/Cessna.pdf.05.02.2009).

Chauzat, M., Martel, A., Cougoule, N., Porta, P., Lachaize, J., Zeggane, S., Aubert, M., Carpentier, P. and Faucon, J. An assessment of honey bee colony matrices, Apis Mellifera (Hymenoptera Apidae) to monitor pesticide presence in continental France. 2011. Environ. Toxicol. Chem. 30, 103e111.

Charreton, M., Decourtye, A., Henry, M., Rodet, G., Sandoz, J. C., Charnet, P. and Collet, C. A locomotor deficit induced by sublethal doses of pyrethroid and neonicotinoid insecticides in the honeybee Apis mellifera. 2015. *PloS one*, *10*(12), e0144879.

Chiesa, L. M., Panseri, S., Nobile, M., Ceriani, F. and Arioli, F. Distribution of POPs, pesticides and antibiotic residues in organic honeys from different production areas. 2018. *Food Additives & Contaminants: Part A*, *35*(7), 1340-1355.

Choudhary, A. and Sharma, D. C. Pesticide residues in honey samples from Himachal Pradesh (India). 2008. *Bulletin of Environmental Contamination and Toxicology*, *80*(5), 417-422.

Commendatore, M., Yorio, P., Scenna, L., Ondarza, P. M., Suárez, N., Marinao, C. and Miglioranza, K. S. Persistent organic pollutants in sediments, intertidal crabs, and the threatened Olrog's gull in a northern Patagonia salt marsh, Argentina. 2018. *Marine pollution bulletin*, *136*, 533-546.

Couvillon, M. J., Schürch, R. and Ratnieks, F. L. W. Waggle dance distances as integrative indicators of seasonal foraging challenges. 2014a. PLoS One 9:e93495.

Crailsheim, K. The Flow of Jelly within a Honeybee Colony. 1992. J Comp Physiol B 162: 681-689. doi:10.1007/BF00301617.

Crailsheim, K., Schneider L. H. W., Hrassnigg, N., Bühlmann, G., Brosch, U., Gmeinbauer, R. and Schöffmann, B. Pollen consumption and utilization in worker honeybees (Apis mellifera carnica): dependence on individual age and function. 1992. J Insect Physiol 38: 409-419. doi:10.1016/0022-1910(92)90117-V.

Davie-Martin, C. L., Hageman, K. J. and Chin, Y. P. An improved screening tool for predicting volatilization of pesticides applied to soils. 2012. *Environmental science & technology*, *47*(2), 868-876.

De Wit, C. A. An overview of brominated flame retardants in the environment. 2002. *Chemosphere*, *46*(5), 583-624.

Decourtye, A., Mader, E. and Desneux, N. Landscape enhancement of floral resources for honey bees in agro-ecosystems. 2010. Apidologie 41:264-277. doi:10.1051/apido/2010024.

Department of Soil Protection. The Netherlands Soil Contamination Guidelines, Netherlands Intervention Values for Soil Remediation, Reference No. DBO/07494013, Amsterdam, The Netherlands; 1994.

Di Rienzo, J. A., Casanoves, F., Balzarini, M. G., Gonzalez, L., Tablada, M. and Robledo, C. W. InfoStat versión 2013. Grupo InfoStat, FCA, Universidad Nacional de Córdoba, Argentina.

Dimond, J. B. and Owen, R. B. Long-term residue of DDT compounds in forest soils in Maine. 1996. *Environmental Pollution*, *92*(2), 227-230.

Eggen, T. and Majcherczyk, A. Effects of zero-valent iron (Fe0) and temperature on the transformation of DDT and its metabolites in lake sediment. 2006. *Chemosphere*, *62*(7), 1116-1125.

Eguchi, A., Isobe, T., Subramanian, A., Sudaryanto, A., Ramu, K., Minh, T. B., Chakraborty, P., Minh, N. H., Tana, T. S., Viet, P. H., Takahashi, S. and Tanabe, S. Contamination by brominated flame retardants in soil samples from open dumping sites of Asian developing countries. 2009. *Organohalogen Compd*, *71*(143), e152.

El-Zoghby, M., Ismail, S.M., Shehata, M. A. and El Saidy, S. Influence of home processing on residues of some organochlorine insecticides in Bouri fish from certain Egyptian lakes. 2000. J.Home Econ. Minuffia Univ. 9, 2133.

Eqani, S.A.; Naseem, R.; Cincinelli, A.; Zhang, G.; Mohammad, A.; Qadir, A.; Rashid, A.; Bokhari, H.; Jones, K.C. and Katsoyiannis, A. Uptake of organochlorine pesticides (OCPs) and polychlorinated biphenyls (PCBs) by river water fish: The case of River Chenab. 2013. Sci. Total Environ. 450–451, 83–91.

Frisch, K. V. Tanzsprache und Orientierung tier Biene. 1965. *Berlin Heidelberg New York: Springer-Verlag, I9b*, *5*, 573.

Fulton, C. A., Hartz, K. E. H., Fell, R. D., Brewster, C. C., Reeve, J. D. and Lydy, M. J. An assessment of pesticide exposures and land use of honey bees in Virginia. 2019. *Chemosphere*.

Gallai, N., Salles, J. M., Settele, J. and Vaissière, B. E. Economic valuation of the vulnerability of world agriculture confronted with pollinator decline. 2009. *Ecological economics*, *68*(3), 810-821.

Ghini, S., Fernandez, M., Pico, Y., Marin, R., Fini, F., Manes, J. and Girotti, S. Occurrence and distribution of pesticides in the province of Bologna, Italy, using honey bees as bioindicators. 2004. Arch. Environ. Contam. Toxicol. 47, 479e488.

Giesy, J. P., Dobson, S. and Solomon, K. R. Ecotoxicological Risk Assessment for Roundup® Herbicide. 2000. Reviews of Environmental Contamination and Toxicology, Vol. 167, pp. 35-120. New York: Springer.

González García, G. Ministerio de salud: Programa Nacional de Riesgos Químicos. 2003. Sustancias prohibidas o restringidas en la república Argentina. 2003- http:// www.msal.gov.ar/htm/site/prog NRQ sustancias.asp

Gonzalez, M., Miglioranza, K. S., Shimabukuro, V. M., Londono, O. M. Q., Martinez, D. E., Aizpún, J. E. and Moreno, V. J. Surface and groundwater pollution by organochlorine compounds in a typical soybean system from the south Pampa, Argentina. 2012. *Environmental Earth Sciences*, *65*(2), 481-491.

Gonzalez, M., Miglioranza, K. S., Grondona, S. I., Barni, M. F. S., Martinez, D. E. and Peña, A. Organic pollutant levels in an agricultural watershed: the importance of analyzing multiple matrices for assessing streamwater pollution. 2013. *Environmental Science: Processes & Impacts*, *15*(4), 739-750.

Gouin, T., Shoeib, M. and Harner, T. Atmospheric concentration of current-use pesticides across south-central Ontario using monthly-resolved passive air samplers. 2008a. Atmos. Environ. 42, 8096e8104.

Grüter, C. and Farina, W. M. The honey bee waggle dance: can we follow the steps?. 2009. *Trends in Ecology & Evolution*, *24*(5), 242-247.

Ham, S. Y., Kim, Y. J. and Lee, D. H. Leaching characteristics of PCDDs/DFs and dioxin-like PCBs from landfills containing municipal solid waste and incineration residues. 2008. *Chemosphere*, *70*(9), 1685-1693.

Hawker, D. D and Connell, D. W. Octanol- water partition coefficients of polychlorinated byphenyl congeners. 1988. Environ. Sci. Technol. 22, 382-385.

Helm, P.A., Bidleman, T.F., Li, H.H. and Fellin, P. Seasonal and Spatial Variation of Polychlorinated Naphthalenes and Non-/Mono-Ortho-Substituted Polychlorinated Biphenyls in Arctic Air. 2004. Environ. Sci. Technol. 38, 5514–5521.

Henry, M., Cerrutti, N., Aupinel, P., Decourtye, A., Gayrard, M., Odoux, J.-F., Pissard, A., Rüger, C. and Bretagnolle, V. Reconciling laboratory and field assessments of neonicotinoid toxicity to honey bees. 2015. Proc. R. Soc. B, 282, 20152110. http://docplayer.es/208714-Plaguicidas-organoclorados.html (accessed on 10 January 2018).

ISTAT. The Distribution of Phytosanitary Products in the Agriculture: Year 2009. 2010. http://www.istat.it/salastampa/comunicati/non_calendario/20101019_ 58 V.H. Estellano et al. / Environmental Pollution 205 (2015) 52e59 00/testointegrale20101019.pdf.

Jergentz, S., Mugni, H., Bonetto, C. and Schulz, R. Assessment of insecticide contamination in runoff and stream water of small agricultural streams in the main soybean area of Argentina. 2005. Chemosphere 61 (6), 817–826.

Johnson, R. M., Pollock, H. S. and Berenbaum, M. R. Synergistic interactions between in-hive miticides in *Apis mellifera*. 2009. *Journal of economic entomology*, *102*(2), 474-479.

Johnson, R. M., Dahlgren, L., Siegfried, B. D. and Ellis, M. D. Acaricide, fungicide and drug interactions in honey bees (*Apis mellifera*). 2013. *PloS one*, *8*(1), e54092.

Keith, L.H., Crumett, W. and Wentler, G. Principles of environmental analysis. 1983. Anal Chem. 1983;55:2210–8.

Kiljanek, T., Niewiadowska, A., Gaweł, M., Semeniuk, S., Borzęcka, M., Posyniak, A. and Pohorecka, K. Multiple pesticide residues in live and poisoned honeybees–Preliminary exposure assessment. 2017. *Chemosphere*, *175*, 36-44.

Kirchner, W. H. Mad-bee-disease? Sublethal effects of imidacloprid ("Gaucho") on the behavior of honey-bees. 1999. Apidologie 30, 422.

Klein, A. M., Vaissiere, B. E., Cane, J. H., Steffan-Dewenter, I., Cunningham, S. A., Kremen, C. and Tscharntke, T. Importance of pollinators in changing landscapes for world crops. 2006. *Proceedings of the royal society B: biological sciences*, *274* (1608), 303-313.

Koch, R. B., Cutkomp, L. K. and Do, F. M. Chlorinated hydrocarbon insecticide inhibition of cockroach and honey bee ATPases. 1969. *Life sciences*, *8*(6), 289-297.

Krupke, C. H., Hunt, G. J., Eitzer, B. D., Andino, G. and Given, K. Multiple routes of pesticide exposure for honey bees living near agricultural fields. 2012. *PLoS one*, *7*(1), e29268.

Kujawski, M. W., Pinteaux, E. and Namieśnik, J. Application of dispersive liquid–liquid microextraction for the determination of selected organochlorine pesticides in honey by gas chromatography–mass spectrometry. 2012. *European Food Research and Technology*, *234*(2), 223-230.

Lambert, O., Piroux, M., Puyo, S., Thorin, C., L'Hostis, M., Wiest, L., Buleté, A. and Pouliquen, H. Widespread occurrence of chemical residues in beehive matrices from apiaries located in different landscapes of Western France. 2013. *PloS one*, *8*(6), e67007.

Lepori, E. C. V., Mitre, G. B. and Nassetta, M. Situación actual de la contaminación por plaguicidas en Argentina. 2013. *Revista Internacional de Contaminación Ambiental*, *29*, 25-43.

Li, J., Zhang, G., Qi, S., Li, X. and Peng, X. Concentrations, enantiomeric compositions, and sources of HCH, DDT and chlordane in soils from the Pearl River Delta, South China. 2006. *Science of the Total Environment*, *372*(1), 215-224.

lolster, P. and Krapovickas, S. Los plaguicidas en uso en la Argentina: riesgos para las aves silvestres. 1999. Temas de Naturalezas y Conservación No. 2, Monografías Asociación Ornitológica del Plata. Buenos Aires: Bird Life International, Aves Argentinas y Embajada Real de los Países Bajos.

Lupi, L., Bedmar, F., Wunderlin, D. A. and Miglioranza, K. S. Organochlorine pesticides in agricultural soils and associated biota. 2016. *Environmental Earth Sciences*, *75*(6), 519.

Malhat, F. M., Haggag, M. N., Loutfy, N. M., Osman, M. A. and Ahmed, M. T. Residues of organochlorine and synthetic pyrethroid pesticides in honey, an indicator of ambient environment, a pilot study. 2015. *Chemosphere*, *120*, 457-461.

Matscheko, N., Tysklind, M., de Wit, C., Bergek, S., Andersson, R. and Sellström, U. Application of sewage sludge to arable land–soil concentrations of polybrominated diphenyl ethers and polychorinated dibenzo-p-dioxins, dibenzofurans, and biphenyls, and their accumulation in earthworms. 2002. *Environmental Toxicology and Chemistry: An International Journal*, *21*(12), 2515-2525.

Medici, S. K., Blando, M., Sarlo, E., Maggi, M., Espinosa, J. P., Ruffinengo, S., Bianchi, B., Eguaras, M. and Recavarren, M. Pesticide residues used for pest control in honeybee colonies located in agroindustrial areas of Argentina. 2019. *International Journal of Pest Management*, 1-10.

Matsumoto, T. Reduction in homing flights in the honey bee Apis mellifera after a sublethal dose of neonicotinoid insecticides. 2013. *Bulletin of Insectology*, *66*(1), 1-9.

Metcalfe, T. L. and Metcalfe, C. D. The trophodynamics of PCBs including mono and non-ortho congeners in the food web of north-Central Lake Ontario. 1997. Sci. Total Environ. 201, 245–272.

Miglioranza, K. S., Aizpún de Moreno, J. E. and Moreno, V. J. Dynamics of organochlorine pesticides in soils from a southeastern region of Argentina. 2003. *Environmental Toxicology and Chemistry: An International Journal*, *22*(4), 712-717.

Miglioranza, K. S., Gonzalez, M., Ondarza, P. M., Shimabukuro, V. M., Isla, F. I., Fillmann, G., Aizpún, J. E. and Moreno, V. J. Assessment of Argentinean Patagonia pollution: PBDEs, OCPs and PCBs in different matrices from the Río Negro basin. 2013. *Science of the Total Environment*, *452*, 275-285.

Mitchell, E. A., Mulhauser, B., Mulot, M., Mutabazi, A., Glauser, G. and Aebi, A. A worldwide survey of neonicotinoids in honey. 2017. *Science*, *358*(6359), 109-111.

MGP (Municipalidad de General Pueyrredón). Dirección General de Gestión Ambiental Ente de Obras y Servicios Urbanos. 2013. "Informe Anual Ambiental 2013". Mar del Plata. Buenos Aires. Argentina. Disponible en http://www.mardelplata.gob.ar/documentos/enosur/informeanual ambiental2013.pdf (visitada el 20 de agosto de 2014).

Mugni, H., Demetrio, P., Marino, D., Ronco, A. and Bonetto, C. Toxicity persistence following an experimental cypermethrin and chlorpyrifos application in pampasic surface waters (Buenos Aires, Argentina). 2010. Bulletin of Environmental Contamination and Toxicology 84, 524-528.

Mullin, C. A., Frazier, M., Frazier, J. L., Ashcraft, S., Simonds, R., vanEngelsdorp, D. and Pettis, J. S. High levels of miticides and agrochemicals in North American apiaries: implications for honey bee health. 2010. PLoS One 5, e9754.

Naug, D. Nutritional stress due to habitat loss may explain recent honeybee colony collapses. 2009. Biol Conserv 142: 2369-2372. doi:10.1016/j.biocon.2009.04.007.

Negoita, T. G., Covaci, A., Gheorghe, A. and Schepens, P. Distribution of polychlorinated biphenyls (PCBs) and organochlorine pesticides in soils from the east Antarctic Coast. 2003. J. Environ. Monit. 5, 281–286.

Neumann, P. and Carreck. N. L. Honey bee colony losses. 2010. *Journal of Apicultural Research*, *49*(1), 1-6.

Nie, H. and Su, S. Differential physiological effects of neonicotinoid insecticides on honey bees: A comparison between Apis mellifera and Apis cerana. 2017. Pestic. Biochem. Physiol. 140, 1–8.

Niell, S., Jesús, F., Pérez, N., Pérez, C., Pareja, L., Abbate, S., Carrasco-Letelier, L., Díaz, S., Mendoza, Y., Cesio, V. and Heinzen, H. Neonicotinoids transference from the field to the hive by honey bees: towards a pesticide residues biomonitor. 2017. *Science of the Total Environment*, *581*, 25-31.

Panseri, S., Catalano, A., Giorgi, A., Arioli, F., Procopio, A., Britti, D. and Chiesa, L. M. Occurrence of pesticide residues in Italian honey from different areas in relation to its potential contamination sources. 2014. Food Control 38, 150e156.

Pareja, L., Colazzo, M., Pérez-Parada, A., Niell, S., Carrasco-Letelier, L., Besil, N., Cesio, M. V. and Heinzen, H. Detection of pesticides in active and depopulated beehives in Uruguay. 2011. *International journal of environmental research and public health*, *8*(10), 3844-3858.

Parker, R. L. The collection and utilization of pollen by the honey bee. 1926. Mem Cornell Agricul Exper Station 98:1–55.

Peng, J., Wu, D., Jiang, Y., Zhang, J., Lin, X., Lu, S., Han P., Zhou J., Li S., Lei Y. and Chen, J. Spatiotemporal variability of polybrominated diphenyl ether concentration in atmospheric fine particles in Shenzhen, China. 2018. Environmental Pollution, 238, 749-759

Potts, S. G., Biesmeijer, J. C., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. E. Global pollinator declines: trends, impacts and drivers. 2010. *Trends in ecology & evolution*, *25*(6), 345-353.

Qiu, X., Zhu, T., Yao, B., Hu, J. and Hu, S. Contribution of dicofol to the current DDT pollution in China. 2005. *Environmental Science & Technology*, *39*(12), 4385-4390.

Rinderer, T. E., Rothenbuhler, W. C. and Gochnauer, T. A. The influence of pollen on the susceptibility of honey-bee larvae to *Bacillus* larvae. 1974. J Invertebr Pathol 23: 347-350. doi:10.1016/0022-2011(74)90100-1. PubMed: 4833177.

Rissato, S. R., Galhiane, M. S., Ximenes, V. F., De Andrade, R. M., Talamoni, J. L., Libânio, M., de Almeida, M., Apon, B. M. and Cavalari, A. A. Organochlorine pesticides and polychlorinated biphenyls in soil and water samples in the Northeastern part of São Paulo State, Brazil. 2006. *Chemosphere*, *65*(11), 1949-1958.

Roulston, T. and Cane, J.H. Pollen nutritional content and digestibility for animals. 2000. Plant Syst. Evol. 222 , 187–209.

Ruiz-Toledo, J., Vandame, R., Castro-Chan, R. A., Penilla-Navarro, R. P., Gómez, J. and Sánchez, D. Organochlorine Pesticides in Honey and Pollen Samples from Managed Colonies of the Honey Bee Apis mellifera Linnaeus and the Stingless Bee Scaptotrigona mexicana Guérin from Southern, Mexico. 2018. *Insects*, *9*(2), 54.

Sakai, S., Ukai, T., Urano, S., Takatsuki, H., Nakamura, K. and Kinoshita, S. Material flow analysis of PCDDs/DFs in a municipal solid waste incineration system. 1998. J. Jpn Soc. Waste Manage. Exp. 9, 123–132 (in Japanese).

SENASA (Animal Health National Service). 2015. [o n l i n e] h t t p : / / w w w. s e n a s a . g o v . a r / contenido.php?to=n&in=1606&io=27155

Silva-Barni, M. F., Gonzalez, M., Wania, F., Lei, Y. D. and Miglioranza, K. S. B. Spatial and temporal distribution of pesticides and PCBs in the atmosphere using XAD-resin based passive samplers: A case study in the Quequén Grande River watershed, Argentina. 2018. *Atmospheric Pollution Research*, *9*(2), 238-245.

Silva-Barni, M. F., Smedes, F., Fillmann, G. and Miglioranza, K. S. B. Passive sampling of pesticides and polychlorinated biphenyls along the Quequén Grande River watershed, Argentina. 2019. *Environmental toxicology and chemistry*, *38*(2), 340-349.

Solomon, K. R., Williams, W. M., Mackay, D., Purdy, J., Giddings, J. M. and Giesy, J. P. Properties and uses of chlorpyrifos in the United States. 2014. In *Ecological Risk Assessment for Chlorpyrifos in Terrestrial and Aquatic Systems in the United States* (pp. 13-34). Springer, Cham.

Sponsler, D. B. and Johnson, R. M. Mechanistic modeling of pesticide exposure: The missing keystone of honey bee toxicology. 2017. *Environmental toxicology and chemistry*, *36*(4), 871-881.

Suchail, S., Guez, D. and Belzunces, L. P. Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in Apis mellifera. 2001. *Environmental toxicology and chemistry*, *20*(11), 2482-2486.

Sundstrom, G., Hutzinger, O. and Safe, S. The metabolism of chlorobiphenyls – A review. 1976. Chemosphere. 5, 267-289.

Thompson, H. M. and Hunt, L. V. Extrapolating from honey bees to bumblebees in pesticide risk assessment. 1999. *Ecotoxicology*, *8*(3), 147-166.

Tomé, H., Ramos, G., Araújo, M., Santana, W., Santos, G., Guedes, R., Maciel, C., Newland, P. and Oliveira, E. Agrochemical synergism imposes higher risk to Neotropical bees than to honeybees. 2017. R Soc Open Sci. 4(1):160866.

Tong, Z., Duan, J., Wu, Y., Liu, Q., He, Q., Shi, Y., Yu, L. and Cao, H. A survey of multiple pesticide residues in pollen and beebread collected in China. 2018. *Science of The Total Environment*, *640*, 1578-1586.

Tosi, S., Burgio, G. and Nieh, J. C. A common neonicotinoid pesticide, thiamethoxam, impairs honey bee flight ability. 2017. Sci. Rep. 7, 1201.

Tremolada, P., Bernardinelli, I., Colombo, M., Spreafico, M. and Vighi, M. Coumaphos distribution in the hive ecosystem: Case study for modeling applications. 2004. *Ecotoxicology*, *13*(6), 589-601.

UNEP. Stockholm Convention. 2011. http://chm.pops.int/Convention/POPsReviewCommittee/POPRCMeetings/POPRC6/tabid/713/mctl/ViewDetails/EventModID/871/EventID/86/xmid/2887/language/es-CO/Default.aspx.

Urlacher, E., Monchanin, C., Rivière, C., Richard, F. J., Lombardi, C., Michelsen-Heath, S., Hageman, K.J. and Mercer, A. R. Measurements of chlorpyrifos levels in forager bees and comparison with levels that disrupt honey bee odor-mediated learning under laboratory conditions. 2016. *Journal of chemical ecology*, *42*(2), 127-138.

Vandame, R.; Meled, M.; Colin, M. E. and Belzunces, L. P. Alteration of the homing-flight in the honey bee Apis mellifera L. exposed to sublethal dose of Deltamethrin. Environ. 1995. Toxicol. Chem. 14, 855–860.

Van Buren, N. W. M., Marin, J., Velthuis, H. H. W. and Oudejans, R.C.H.M. Residues in beeswax and honey of perizin, an acaricide to combat the mite Varroa jacobsoni Oudemans (Acari: Mesostigmata). 1992. Environ. Entomol. 21(4), 860–5.

Wahl, O. and Ulm, K. Influence of pollen feeding and physiological condition on pesticide sensitivity of the honey bee *Apis mellifera carnica*. 1983. Oecologia 59: 106-128. doi:10.1007/BF00388082.

Wang, J., Kliks, M. M., Jun, S. and Li, Q. X. Residues of organochlorine pesticides in honeys from different geographic regions. 2010. *Food research international*, *43*(9), 2329-2334.

Williamson, S. M. and Wright, G.A. Exposure to multiple cholinergic pesticides impairs olfactory learning and memory in honey bees. J. Exp. Biol. 2013. 216, 1799–1807.

Wu, J. Y., Anelli, C.M. and Sheppard, W.S. Sub-lethal effects of pesticide residues in brood comb on worker honey bee (Apis mellifera) development and longevity. 2011. PLoS ONE, 6, e14720.

Yao, Y., Tuduri, L., Harner, T., Blanchard, P., Waite, D., Poissant, L., Murphy, C., Belzer, W., Aulagnier, F., Li, Y.-F. and Sverko, E. Spatial and temporal distribution of pesticide air concentrations in Canadian agricultural regions. 2006. Atmos. Environ. 40, 4339e4351.

Yao, Y., Harner, T., Ma, J., Tuduri, L. and Blanchard, P. Sources and occurrence of dachtal in the Canadian atmosphere. 2007. Environ. Sci. Technol. 41, 688e694.

Yu, H. Y., Bao, L. J., Liang, Y. and Zeng, E. Y. Field validation of anaerobic degradation pathways for dichlorodiphenyltrichloroethane (DDT) and 13 metabolites in marine sediment cores from China. 2011. *Environmental science & technology*, *45*(12), 5245-5252.

Yu, H. Y., Guo, Y., Bao, L. J., Qiu, Y. W. and Zeng, E. Y. Persistent halogenated compounds in two typical marine aquaculture zones of South China. 2011. *Marine pollution bulletin*, *63*(5-12), 572-577.

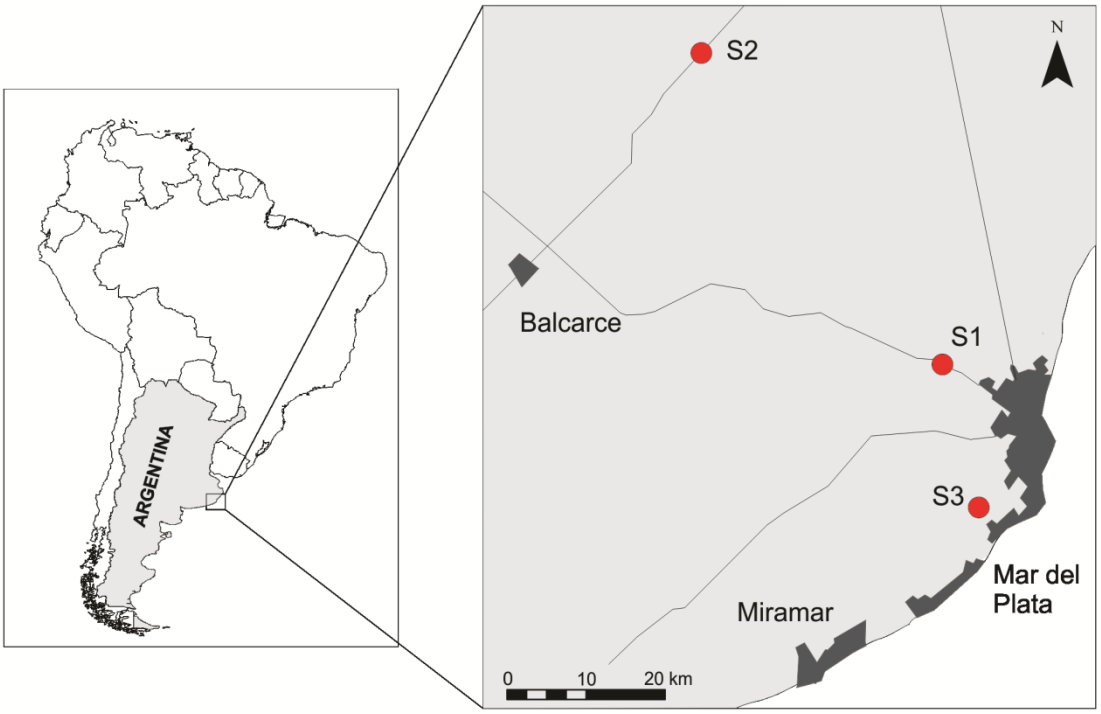

Fig. 1. Study area showing sampling sites of honey bees, bee bread, honey, soil and flowers. S1: farm settled inside a fruit-horticultural belt. S2: agricultural field dedicated to extensive soybean production. S3: field adjacent to urban disposal waste.

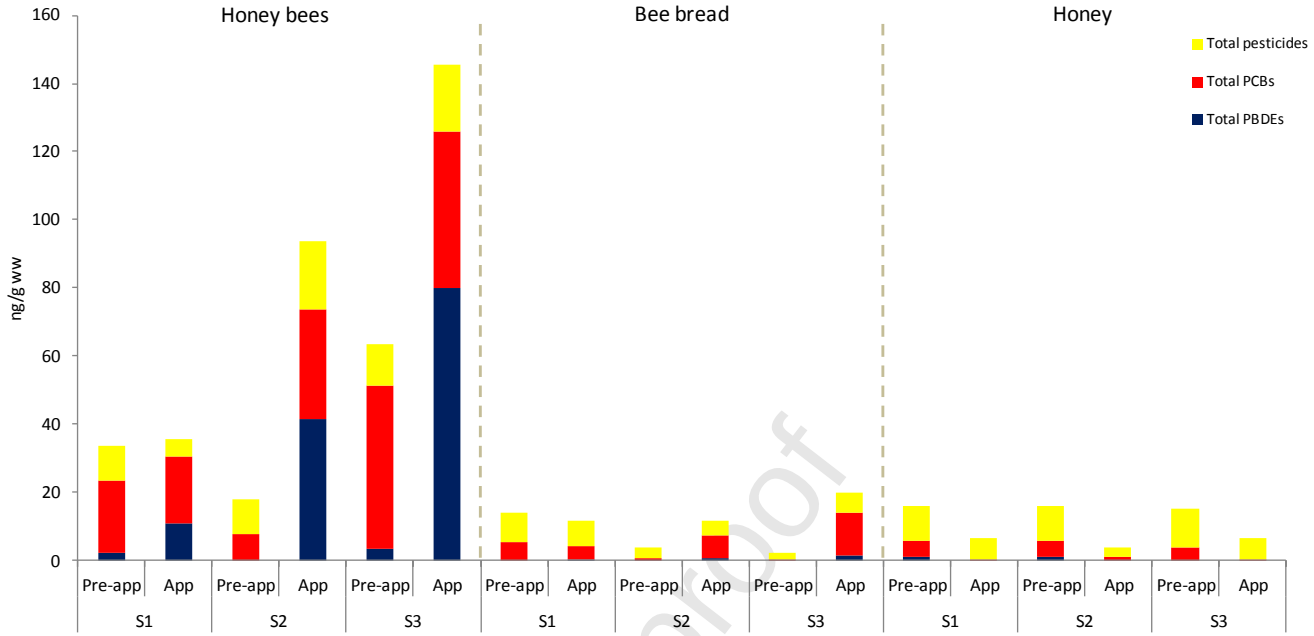

Fig. 2. Distribution of total contaminants (pesticides, PCBs and PBDEs) in honey bees, bee bread and honey samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

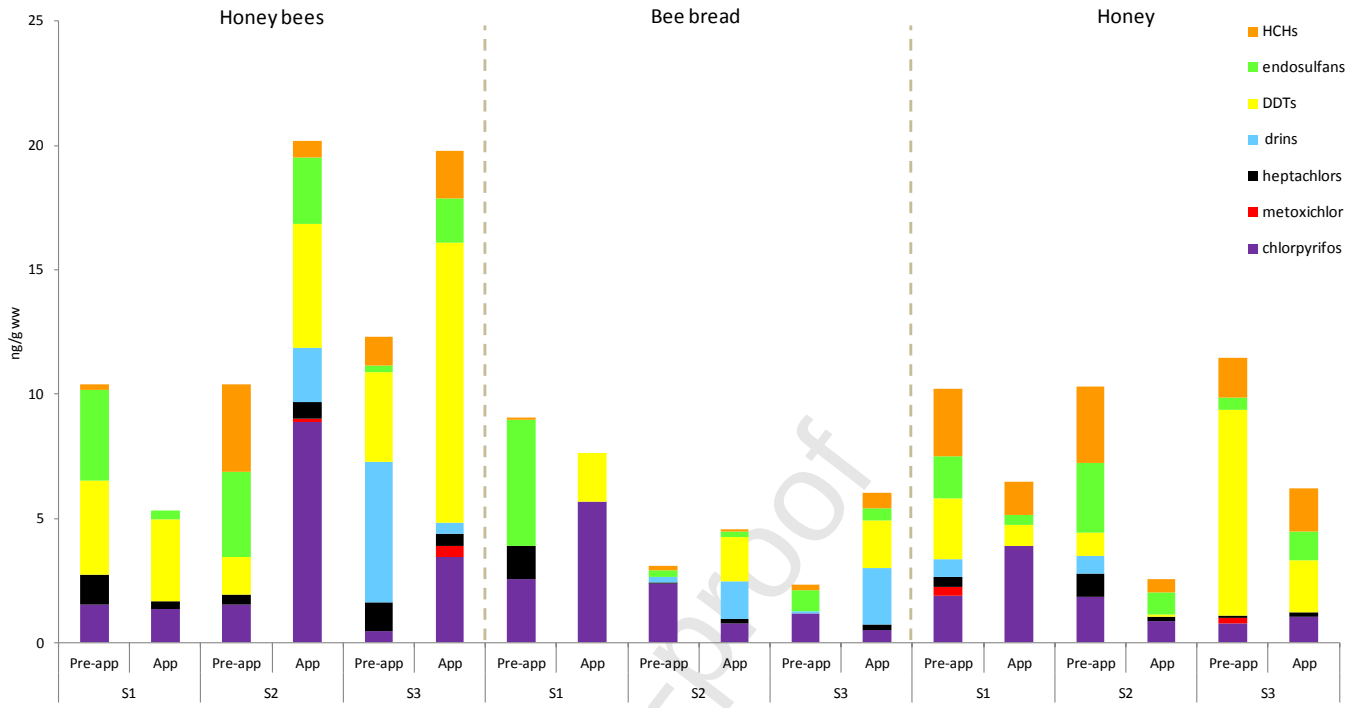

Fig. 3. Distribution of pesticides in honey bees, bee bread and honey samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

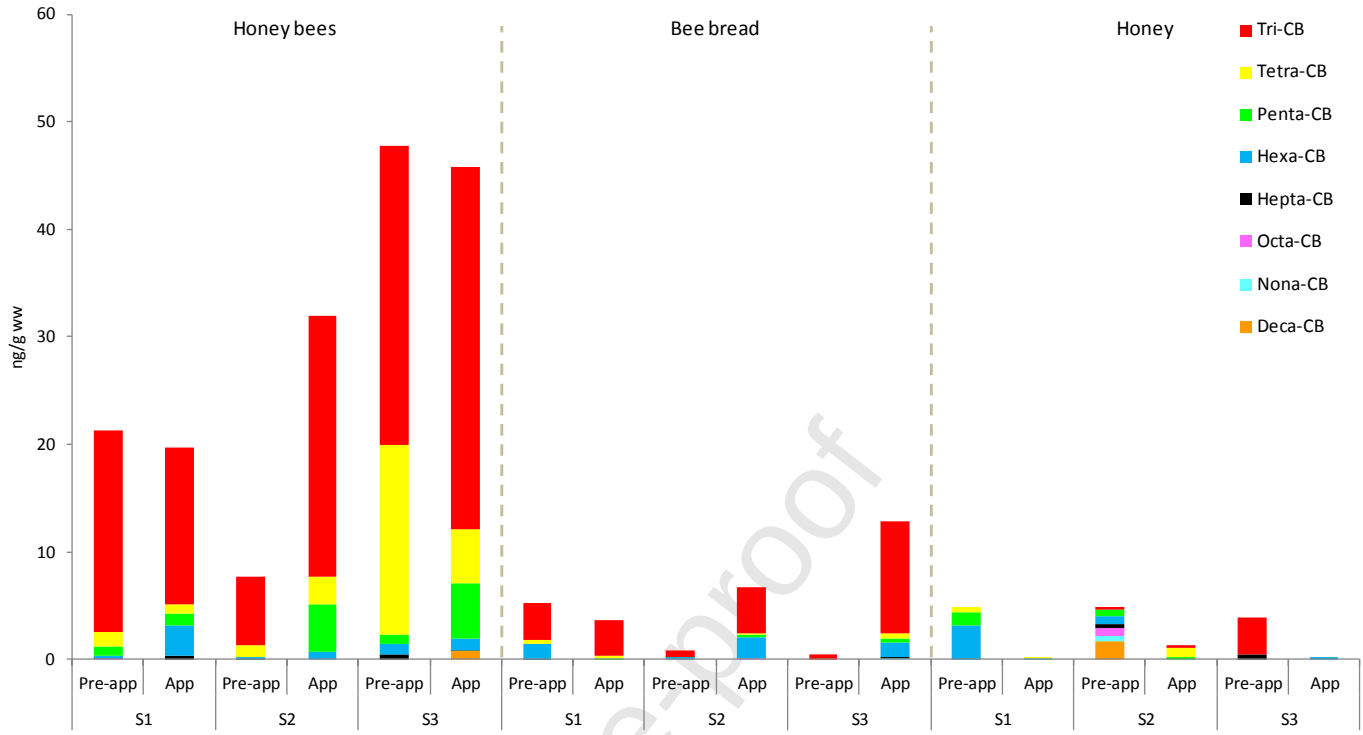

Fig. 4. Distribution of groups of PCBs in honey bees, bee bread and honey samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

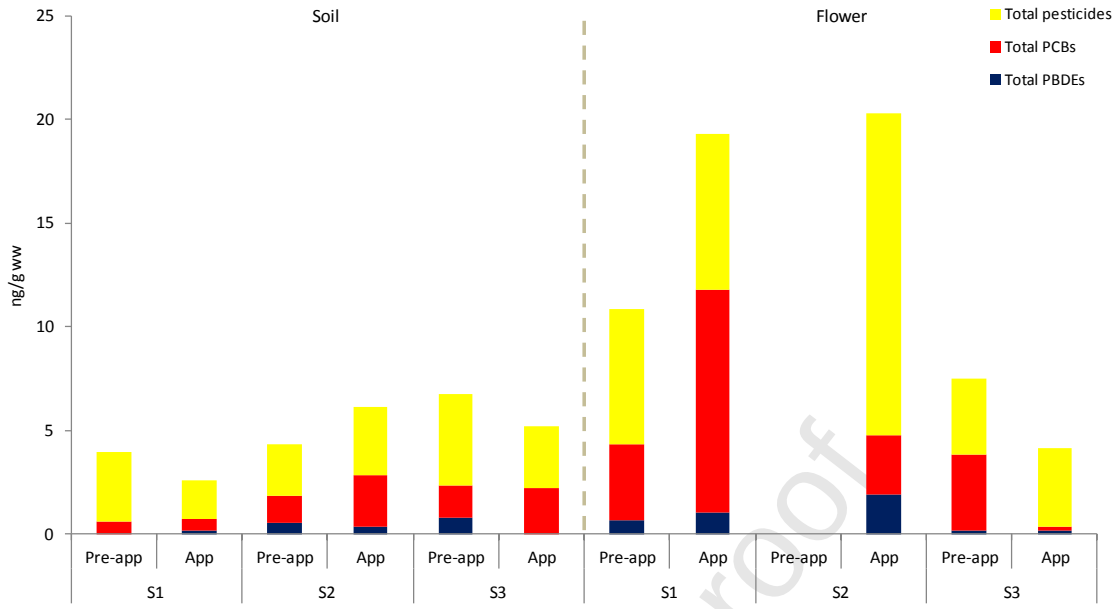

Fig. 5. Distribution of total contaminants (pesticides, PCBs and PBDEs) in soil and flower samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

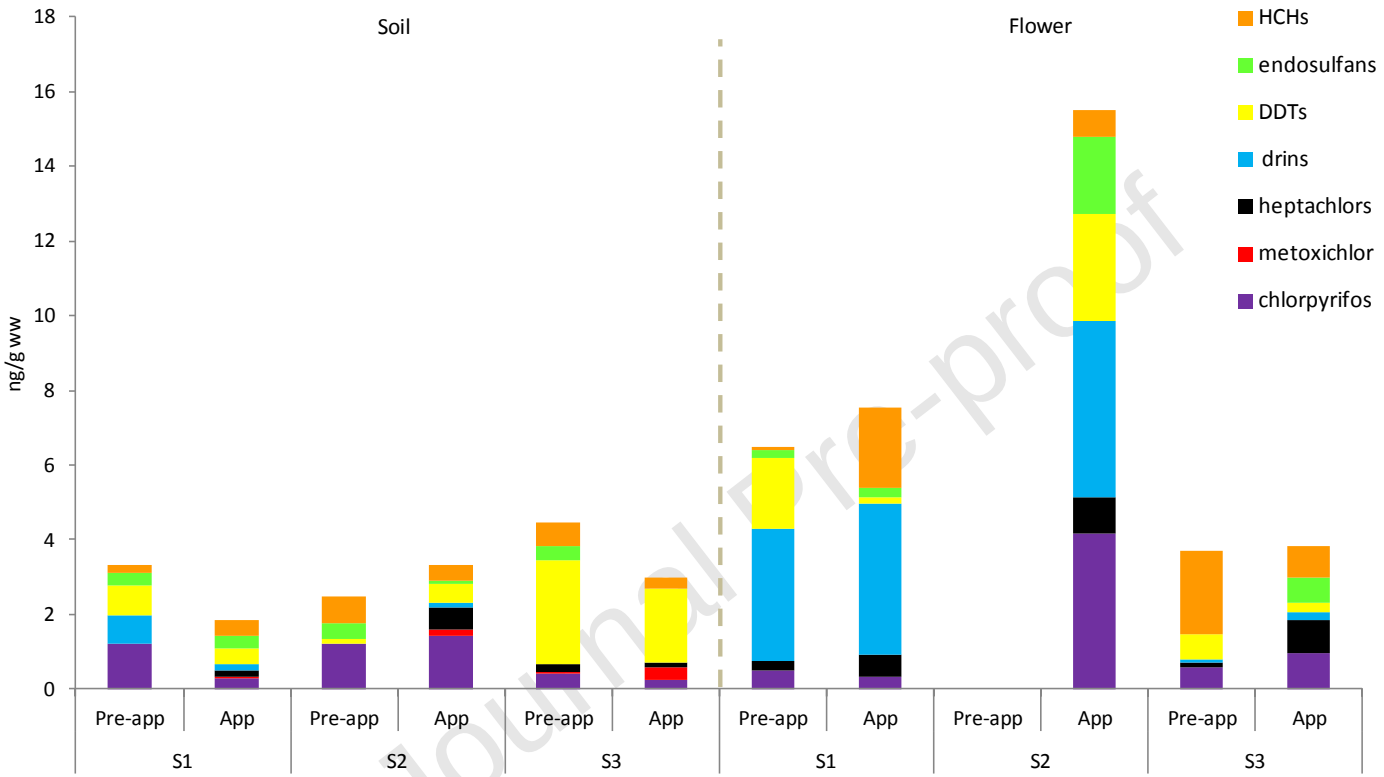

Fig. 6. Distribution of pesticides in soil and flower samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

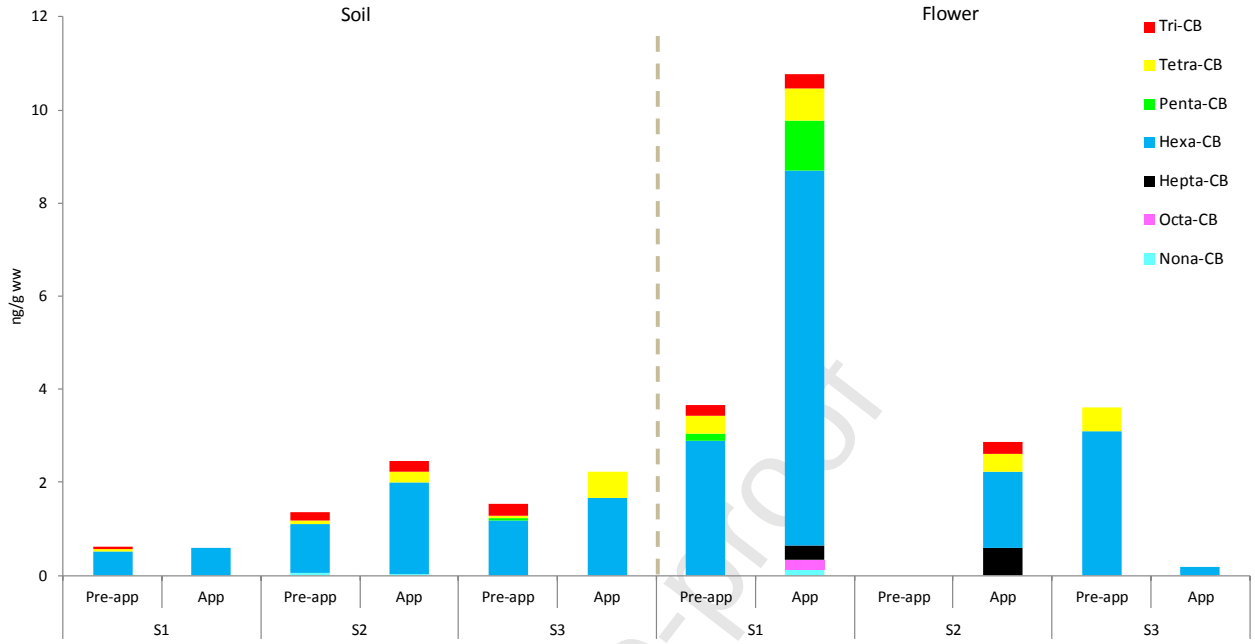

Fig. 7. Distribution of groups of PCBs in soil and flower samples from a farm settled inside a fruit-horticultural belt (S1), in an agricultural field dedicated to extensive soybean production (S2), and in a field adjacent to urban disposal waste (S3) during pre-application (Pre-app, Period 1) and application (App, Period 2) periods of pesticides.

Graphical abstract

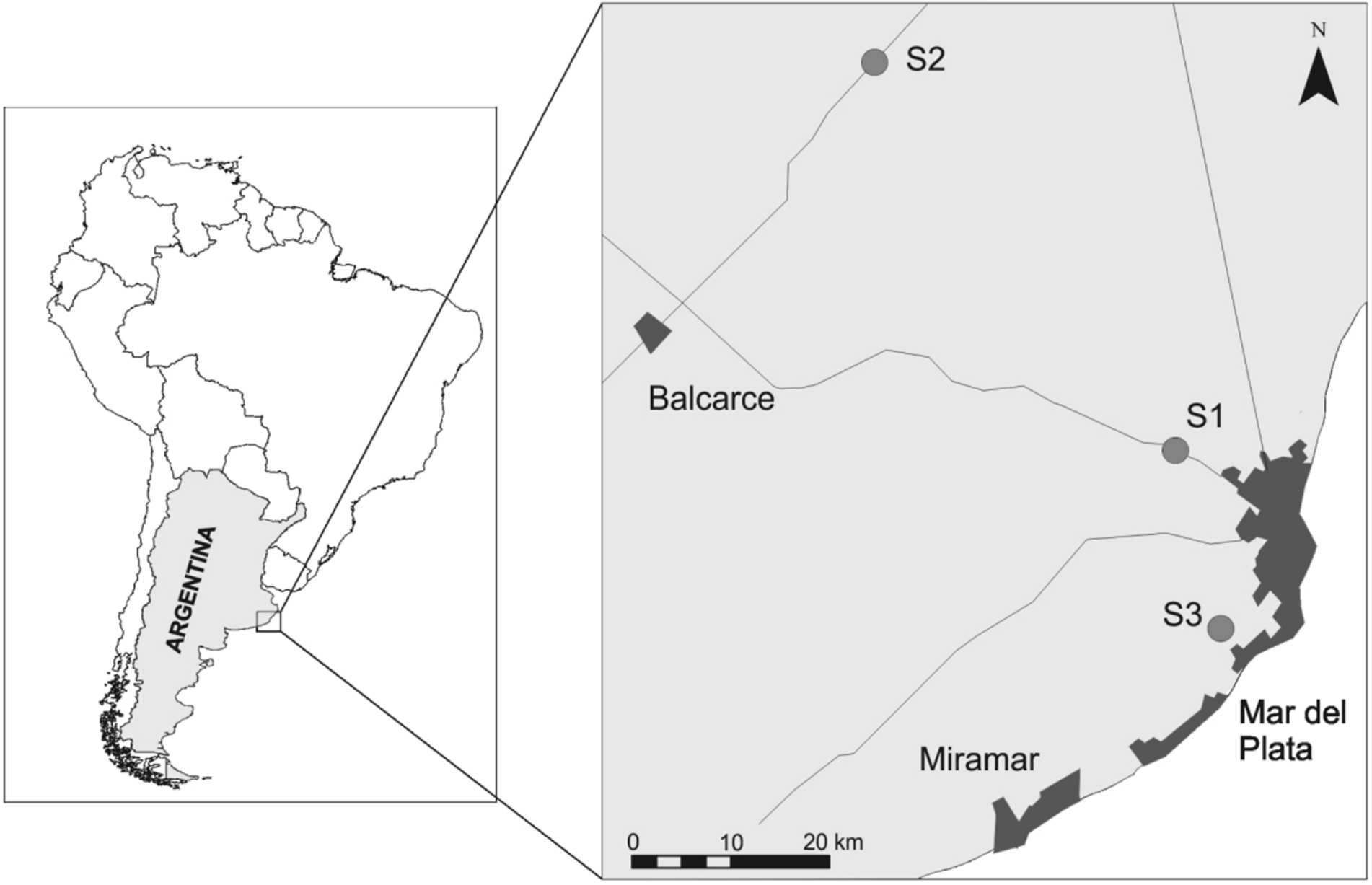

Figure 1

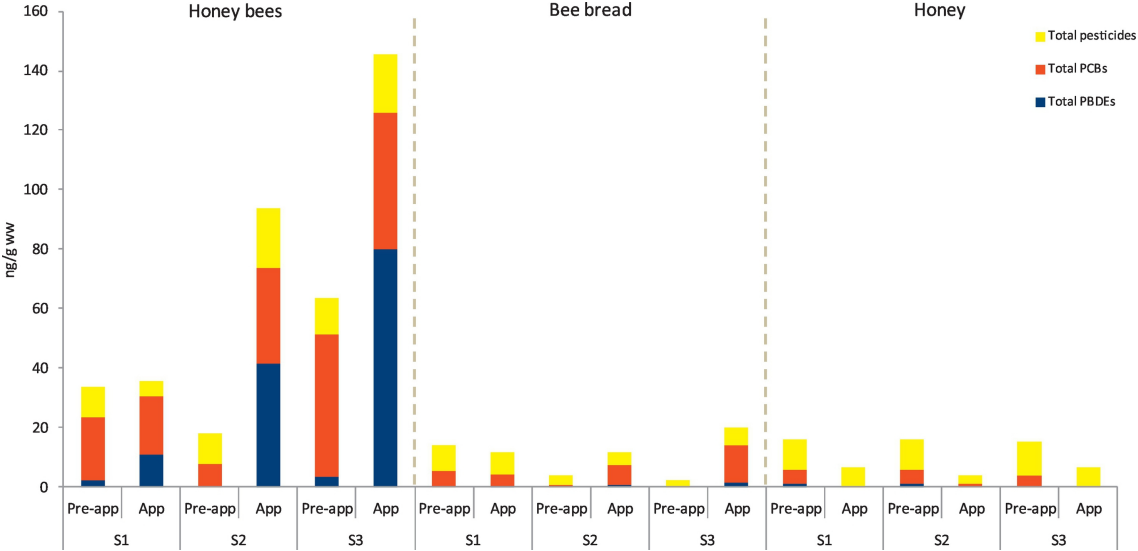
Honey bees
Bee bread
Honey
Total pesticides
Total PCBs
Total PBDEs
ng/g ww
160
140
120
100
80
60
40
20
0
Pre-app
App
S1
S2
S3

Figure 2

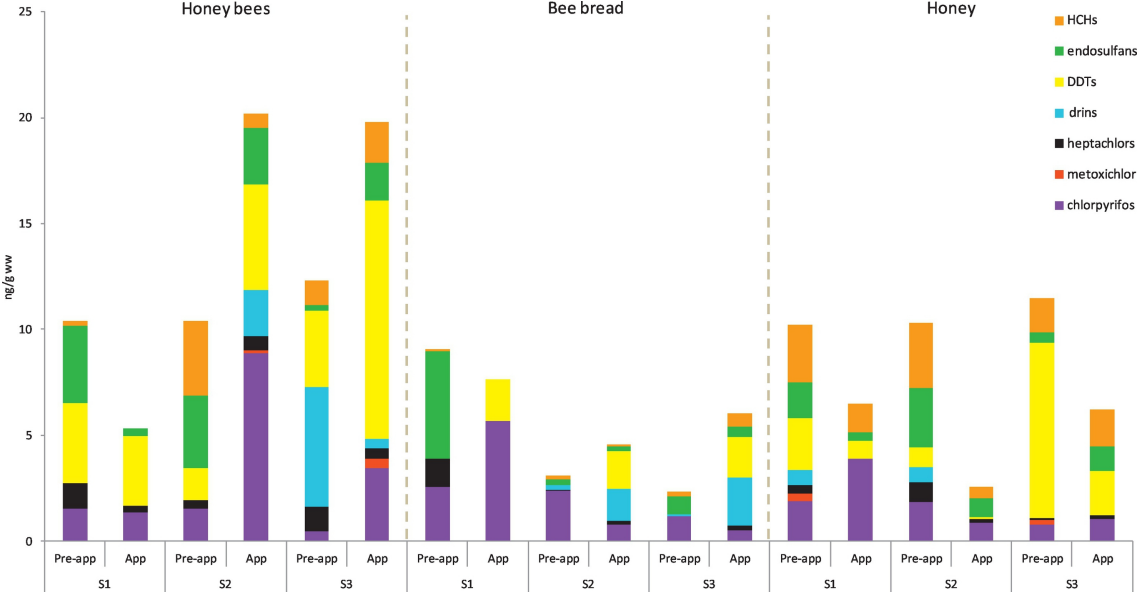
Honey bees
Bee bread
Honey
HCHs
endosulfans
DDTs
drins
heptachlors
metoxichlor
chlorpyrifos
ng/g ww
25
20
15
10
5
0
Pre-app
App
S1
S2
S3

Figure 3

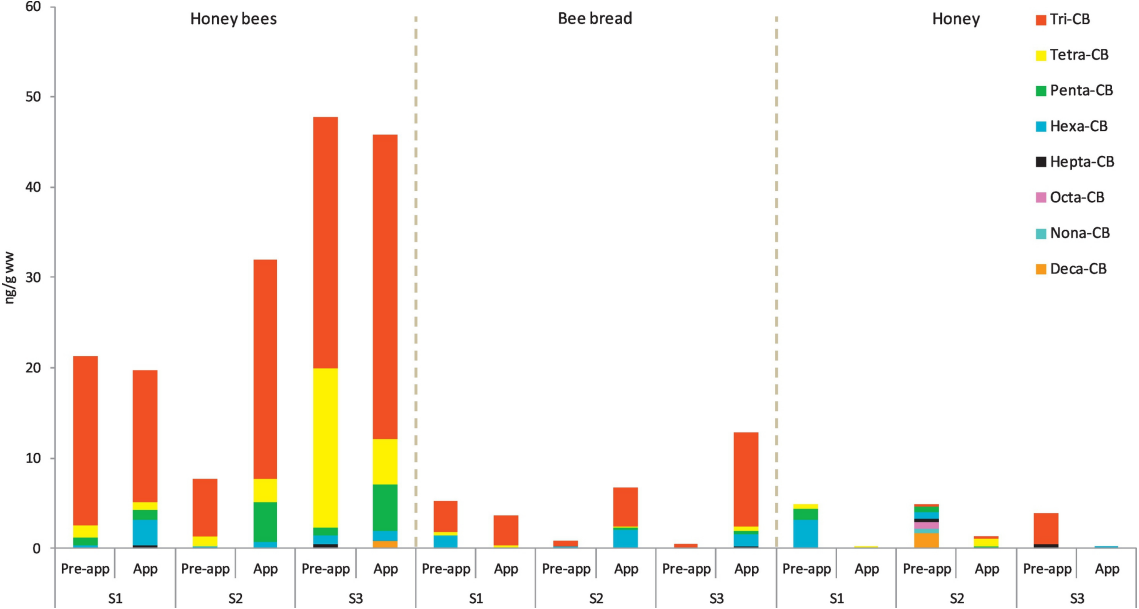
Honey bees
Bee bread
Honey
Tri-CB
Tetra-CB
Penta-CB
Hexa-CB
Hepta-CB
Octa-CB
Nona-CB
Deca-CB
ng/g ww
60
50
40
30
20
10
0
Pre-app
App
S1
S2
S3

Figure 4

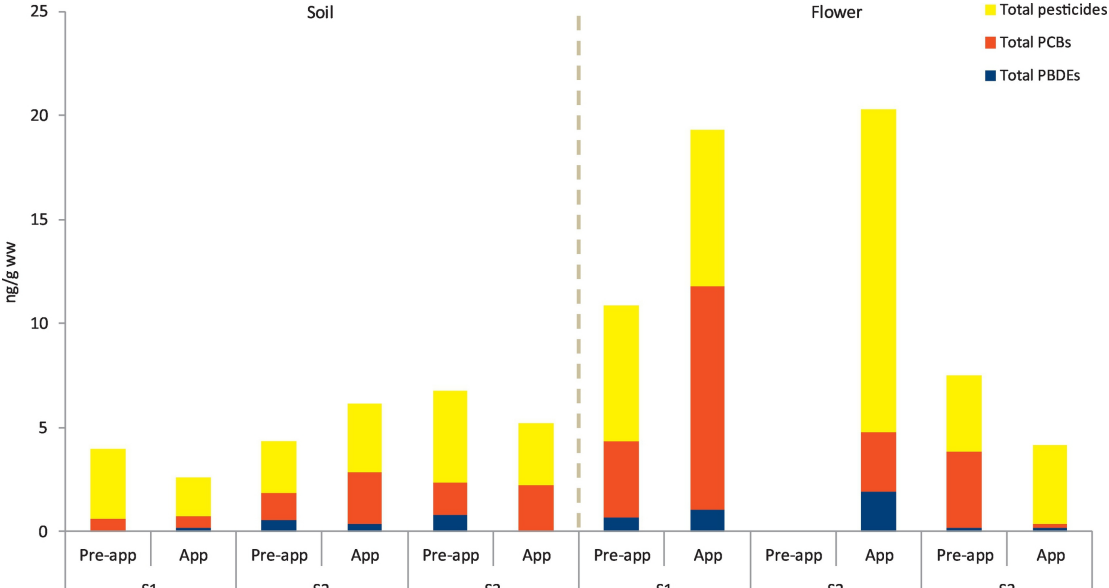
Soil
Flower
Total pesticides
Total PCBs
Total PBDEs
ng/g ww
25
20
15
10
5
0
Pre-app
App
S1
S2
S3

Figure 5

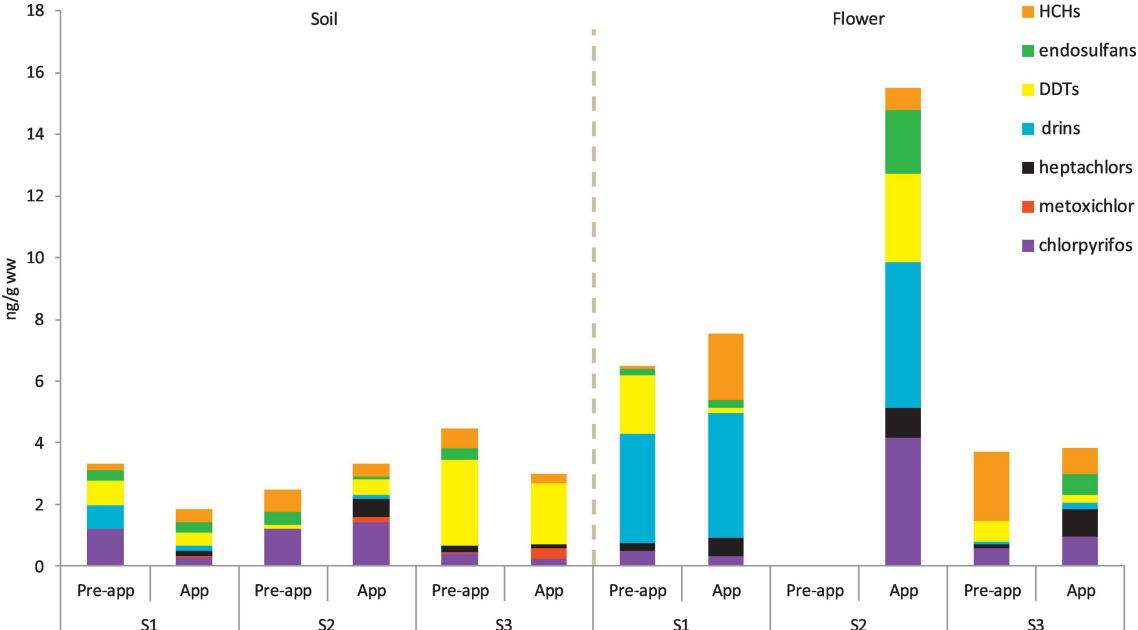
Soil
Flower
HCHs
endosulfans
DDTs
drins
heptachlors
metoxichlor
chlorpyrifos
ng/g ww
18
16
14
12
10
8
6
4
2
0
Pre-app
App
S1
S2
S3

Figure 6

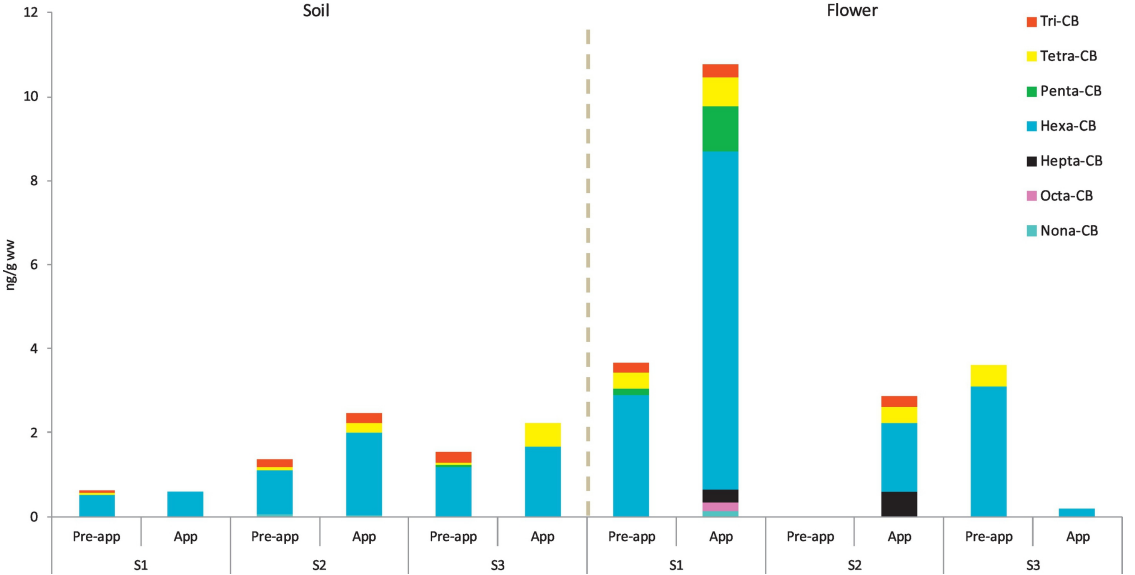
Soil
Flower
Tri-CB
Tetra-CB
Penta-CB
Hexa-CB
Hepta-CB
Octa-CB
Nona-CB
ng/g ww
12
10
8
6
4
2
0
Pre-app
App
S1
S2
S3

Figure 7